



Perspectives of Dendrimer-based Nanoparticles in Cancer Therapy

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ABSTRACT

Currently, cancer is the second most common cause of death in the United States, exceeded only by heart disease. Chemotherapy traditionally suffers from a non-specific distribution, with only a small fraction of the drug reaching the tumor, in this sense, the use of dendrimers incorporating drugs non-covalently encapsulated inside the dendrimer or covalently conjugated have proven to be effective against different cancer cell lines. However, at present the dendrimers used as drug-carriers still do not meet the necessary characteristic to be considered as an ideal dendrimer for drug delivery; high toxicity, bio-degradability, low toxicity, biodistribution characteristics, and favorable retention with appropriate specificity and bioavailability have not been fully covered by the current available dendrimers. However, the development and study of new dendrimers drug-carriers continues to be an important tool in the cancer therapy as they can be functionalized with varied ligands to reach the tumor tissue through the different body barriers in the body with minimal loss of activity in the bloodstream, have the ability to selectively kill tumor cells without affecting the normal cells and most important with a release mechanism controlling actively. Given the continuous efforts and research in this area of interest, we presented in this review the work done with a special emphasis on the development of dendrimers as a major tool in the combination with drugs, as a potential adjunctive agent in anticancer therapy.

Key words: dendrimers, cancer therapy, pharmacodynamics, nanoparticles.

INTRODUCTION

Cancer is a major cause of deaths and is expected to become a major source of morbidity and mortality during coming decades. In 2012, an approximated

of 14.1 million people were diagnosed with cancer, and about 8.2 million people died of cancer (Ferlay et al. 2015). Currently, cancer is the second most common cause of death in the United States, with lung cancer, liver cancer and stomach cancer being the most common cause of death, exceeded only by heart disease (American Cancer Society 2011).

Cancer is an uncontrolled cell proliferation caused by physical, chemical, genetic or biological

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factors. There are dozens of ways in which the disease occurs but its basic pathophysiology comprises aberrations at any point in the molecular machinery that governs the cell cycle and therefore cause the deregulations of this. The progression at a genetic level is the accumulation of successive mutations, since a single somatic mutation is not enough to develop a cancer. In order for a tumor to appear, it is necessary to lose the multiple controls exerted by the three gene categories: oncogenes, cancer suppressor genes and apoptosis regulatory genes; the accumulation of these mutations is a consequence of the genetic instability of the abnormal cells (Alberts 2004).

Among the proteins that regulate the cell cycle and that are altered can be mentioned the Cyclin D, which has been increased in multiple types of cancer, such as stomach or esophagus (Golias et al. 2004), Cyclin B increases in cases of human papillomavirus (HPVs, human papilloma-viruses) 16 or 18, which are the main cause of cervix cancer in women, the oncoprotein E7, which, like the cyclin D / CDK complex, can block the function of the retinoblastoma protein, a tumor suppressor protein that is altered in many types of cancer by promoting the cell cycle (Kim and Zhao 2005), Cells overexpressing c-myc are resistant to growth arresting effects promoted by the transforming growth factor β (TGF β) which induces the expression of p15, p21 and p27 (the same c-myc repressed CKIs). This situation is also found in approximately 80% of cervical tumors (Gartel and Shchors 2003), and the p53 gene is mutated in half of the known human cancers (of liver, skin, lung, etc.). Myeloid leukemias are part of the other fifty percent where there are no mutations in this gene (Ryan et al. 2001).

Nowadays, targeted therapy is gaining importance due to its specificity towards cancer cells while sparing toxicity to off-target cells. Targeted therapy involves developing drugs that block cancer cell proliferation, promote cell cycle

regulation or induce apoptosis or autophagy and targeted delivery of toxic substances specifically to cancer cells to destroy them (Padma 2015, Gerber 2008). These highly specific agents should be capable of exerting its effect on individual proteins or pathways, either over-expressed or aberrant in tumors, this specificity to eliminate tumor cells is important to avoid toxicities associated with traditional chemotherapies, while resulting in improved antitumor efficacy (Blanco et al. 2011).

Chemotherapy traditionally suffers from a non-specific distribution, with only a small fraction of the drug reaching the tumor, from this point of view the pharmacokinetic properties known as absorption, distribution, metabolism and elimination (ADME), determine the amount of the drug and/or its active metabolite reaching the tumor (Damia and Garattini 2014). The ADME are mediated by drug-metabolizing enzymes and transporters expressed in different tissues including small intestine, liver and kidney. In particular, xenobiotic-metabolizing enzymes such as cytochrome P450 isoforms play a critical role in the metabolic elimination of drugs, and transporters such as ATP binding cassette (ABC) and solute carrier (SLC) transporters have high impact on drug absorption, distribution and excretion. The interactions between drugs and enzymes/transporters ultimately determine the pharmacokinetic properties and subsequently influence the pharmacodynamics (Ai-Ming and Yu-Zhuo 2012).

Today, it is well known that injectable materials undergo sequestration by the mononuclear phagocyte system (MPS), which is a system composed of monocytes and macrophages and organs like the liver, spleen, lungs and bone marrow. Indeed, once in the bloodstream, surface non-modified nanoparticles are rapidly opsonized and massively cleared by the fixed macrophages of the MPS organs (Gustafson et al. 2015, Peer et al. 2007). As a result, the drugs are accumulated in healthy organs, with its inherent toxicity, drawing a

fine line between tolerability and severe morbidity, as is the case of doxorubicin, a DNA intercalating drug that lead to cardiotoxicity (Olson and Mushlin 1990).

In addition, cancer cells maintain a unique pH gradient, creating a unique environment around them, it is more acidic extracellularly and more alkaline intracellularly. The tumor microenvironment increases tumor's fitness by blunting the immune system, activating endogenous immunosuppressive strategies and inhibiting the growth of the normal cell population. Moreover, the tumor microenvironment disables the activities of several chemotherapeutic agents resulting in resistance and failure in drug response either through disturbing drug partitioning, sequestering it intracellularly or through induction of multidrug resistance expression (Alfarouk et al. 2015). All these factors prevent the healing potential of the anticancer drugs, justifying the search for more effective ways to release the drugs into the tumor (Blanco et al. 2011).

With these limitations in mind, Paul Ehrlich coined the term "magic bullet" to chemotherapy (Strebhardt and Ullrich 2008). There are many different types of nanoparticles used in medical therapy as for example nanoparticles of $\text{Fe}_4[\text{Fe}(\text{CN})_6]$ (Long et al. 2016), nanotubes (Li et al. 2017), liposomes, polymer-drug conjugates and others. The liposomes and polymer-drug conjugates were developed in the '60s and '70s (Duncan 2003); these are now the main platforms in the field of nanomedicine (Ferrari 2005), emerging as a research area, which could have profound effects on the current paradigms that exist in various disease states. The scientific community has embraced the possibilities of nanomedicine in diseases such as cancer, whose optimal treatment has eluded researchers for decades due to the use of highly toxic compounds which are non-specific to cancer cells, resulting in an excessive toxicity to the surrounding healthy cells and, for many

patients, the cancerous cells are discovered only after they have spread too extensively for treatment to significantly improve life expectancy and quality of life (LaRocque et al. 2009).

Given the continuous efforts and research in this area of interest, we presented in this review the work done with a special emphasis on the development of dendrimers as a major tool in the combination with drugs, as a potential adjunctive agent in anticancer therapy.

DENDRIMERS

Dendrimers or dendritic molecules correspond to molecules with a central core and repeated branches (Figure 1). They can be classified by its form as polymers, hyperbranched polymers or brush-polymers and also can be classified by their molecular weight as low or high molecular weight (Morikawa 2016).

Its structure provides unique opportunities by chemical conjugation (dendrimer-drug), the supramolecular structure of dendrimer-drug complexes is formed based on different interactions as electrostatic and hydrophobic/hydrogen-bond, among others, or encapsulation, within the central cavity and/or within the multiple channels between the dendrons (Blanco et al. 2011, Lee et al. 2005). The applications of these molecules is quite broad, ranging from drug delivery applications, wherein dendrimer nanoparticles are combined with therapeutics and targeted to specific tissues or gene delivery which works similarly to drug delivery but combining the dendrimer nanoparticles with nucleic acids (Svenson 2009), providing advanced and alternative solutions, as a drug carrier due to the specific physicochemical characteristics, such as polarity, net charge, solubility in water, among others (Satsangi et al. 2015).

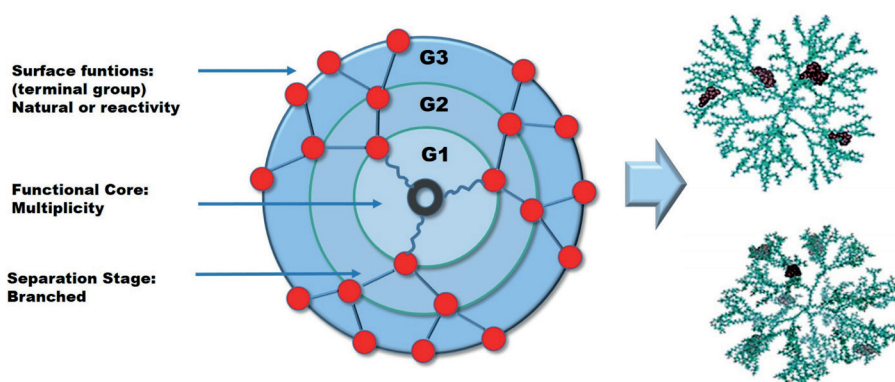


Figure 1 - Architectural components of dendrimers.

STRUCTURE

These molecules are characterized by techniques such as Nuclear magnetic resonance (NMR) of proton (^1H) and carbon (^{13}C), Matrix Assisted Laser - Desorption Ionization - Mass Spectrometry (MALDI-MS), Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC), Scanning electron microscope (SEM), Transmission electron microscopy (TEM), rheology and scattering techniques (Valdes et al. 2016) and have attracted much attention because of their structures, as highly branched macromolecules, possessing symmetrical architecture with a globular shape, in which, all the bridges emerge radially from a core point with a regular branching pattern and with repeated units, each of which contributes to a new branching point (Frechet 2002, Urzua et al. 2016).

Two synthetic methodologies have been used for dendrimers, using reactions of amides formation, esterification and nucleophilic addition: the divergent approach method (Newkome et al. 1985, Tomalia et al. 1985), described by Tomalia et al. (1986), in which the growth begins in the core and proceeds radially toward the periphery of the dendrimer, and the convergent approach described by Hawker (Grayson and Frechet, 2001, Hawker and Frechet 1990), where the dendrimer grows from the periphery to the indoor. The two approaches are complementary and neither of them

generally is better than the other, the choice of the synthetic method used has usually been justified by the characteristics of the target molecule, the chemistry available for growth, and the specific building blocks used in the construction of the dendrimer (Frechet 2002).

The branching units are described by generation, beginning with the central core as generation (-0.5), this is the intermediate generation that then is transformed into the generation 0 (G0) and increases with each successive addition of the branch points (G1, G2, etc); each successive generation increases the number of terminal groups exponentially. Thus, the G5 dendrimer has four generations on branch points emanating from the central core. The dendritic macromolecules increase linearly in diameter, adopting a globular shape with each increased generation of the dendrimer (Sampathkumar and Yarema 2005, Svenson and Tomalia 2005, Wolinsky and Grinstaff 2008).

NANOPARTICLE INTERACTION WITH CELL MEMBRANES AND BIOCOMPATIBILITY

The interaction of nanoparticles and biological membranes is a complex process, difficult to understand in detail due the heterogeneity of both. Despite this obstacle, there is strong interest in discovering the general principles that govern the interactions and the specific details between polymers and particular cell types, offering new

applications of nanoparticles for drug and gene delivery (Leroueil et al. 2007).

Many ideas have been raised about the size of the dendrimer and the ability of terminal groups associated with lipid molecules, based on the assembly of dendrimer-lipid vesicles. The radius of the lipid heads (L) in contact with the surface of the dendrimer and the number of terminal groups of the dendrimer (P) appear to be factors in the formation of the pore. For example, poly amide amine (PAMAM) generation 7 (G7) dendrimers supports low ratios (L/P) allowing the formation of stable vesicles dendrimer-lipid (Mecke et al. 2005).

In separate studies, it was shown that cationic PAMAM dendrimers leads to the formation of a single pore in the membrane fluid phase, while the existence of a gel phase in the plasma membrane is not affected by the presence of these dendrimers, suggesting that depending on the phase of the lipid bilayer could impact in cellular uptake studies under experimental conditions such as size, chemical functionality, and charge of the dendrimer (Mecke et al. 2005, Wolinsky and Grinstaff 2008).

The concept of architecture of the dendrimer and the creation of pores in the lipid bilayer was expanded to a range of linear and dendritic polymers, commonly polycationic dendrimers investigated in *in vitro* drug delivery applications, including poly-L-lysine (PLL), polyethyleneimine (PEI) diethylaminoethyl dextran (DEAE-DEX), and PAMAM, which were compared against neutrally charged polymers, including polyvinyl alcohol (PVA) and polyethylene glycol (PEG), *in vitro*. In this regard, the charge density of the polymers was found to have a significant impact on the permeability of the membrane, with the most densely loaded polymer (PEI), releasing the biggest amount of cytosolic enzymes outside the cell, as well as providing transport of supravital dye. The ability of PAMAM to increase cell membrane permeability was attributed to the spherical architecture necessary to promote interactions between dendrimers and

lipid molecules. Meanwhile, PVA and PEG had no impact on membrane permeability (Hong et al. 2006, Wolinsky and Grinstaff 2008).

Seib et al. (2007) studied the effect of the structure in the cellular mechanism of absorption velocity in linear and branched PEI's, and PAMAM dendrimers (G2, G3 and G4). The G4 PAMAM dendrimers showed the strongest rate of absorption, followed distantly by the branched PEI, PEI linear, G3 and G2 PAMAM in a decreasing magnitude of internalization. The G4 PAMAM and branched PEI were internalized mainly by cholesterol-dependent pathways, whereas the absorption of linear PEI, was independent of cholesterol and clathrin pathways, suggesting that the dendrimer architecture somehow affect cellular internalization (Seib et al. 2007, Wolinsky and Grinstaff 2008).

For its part, the amino groups of some dendrimers influence the biological membranes due to the electrostatic interactions, because of its high positive charges and negative charges of cell membranes (Niederhafner et al. 2008, Tomalia et al. 2007). However, older generations of dendrimers terminated in amino groups (over G3) causes destructive interactions with membranes, which lead to cell lysis and high toxicity. On the other hand, the negatively charged dendrimers (surfaces with carboxylic acids) do not interact with most cell membranes and thus do not have a generation-dependent toxicity. When uncharged dendrimers are studied, their cytotoxicity depends on the polarity of the surface of the terminal groups. Dendrimers, with polar membranes non-invasive as PEG groups have a non-toxic behavior. In contrast, non-polar groups such as lipids interact with the cell membrane by hydrophobic interactions; this results, in some cases to cytotoxic dendrimers. Lipids (especially glycosphingolipids) may also have a positive influence, because they can provide the dendrimers with immunostimulatory properties. The biodistribution, clearance, and toxicity are

closely related to particle size of the nanoparticles (Sebestik et al. 2011).

In general, one can observe that the toxicity of dendrimers is dependent on the chemistry of the core, but is more strongly influenced by the nature of the surface of the dendrimer. For example, the cytotoxicity measured by the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay (MTT assay) of a library of dendrimers based on melamine surfaces, including amines, guanidines, carboxylate, sulfonate, phosphonate, or PEGylated, showed that cationic dendrimers were much more cytotoxic than anionic dendrimers or PEGylated (Chen et al. 2004). Similarly, quaternaries PAMAM-OH showed lower levels of cytotoxicity than PAMAM-NH₂, given that the inner cationic charges were sealed by the surface of the hydroxyl groups (Lee et al. 2003). The surface modification of G4 PAMAM with lysine or arginine leads to increased toxicity compared to G4 PAMAM native, when were incubated in HepG2 cells or human embryonic kidney cells 293 (Choi et al. 2004), this was attributed to increased charge density and molecular size. It has also been found that anionic PAMAM dendrimers G1.5 - 9.5 and dendrimers DAB with surfaces -COOH are not cytotoxic in HepG2, CCRF and B16F10 cells, at a concentration of up to 5 mg/mL (72 h, MTT assay). SEM and B16F10 cells exposed to these anionic dendrimers showed no morphological changes (Malik et al. 2000). A potential toxicity of the dendrimer core is achieved when these are presented to cells with low generation dendrimers, due to its open molecular structure. In addition, low generation dendrimers, have a surface more accessible to end groups, which in the older generation is sterically hindered due to the high accumulation of terminal groups on the surface, the increase in branching (generation) and high surface coverage with biocompatible end groups like PEG are being widely used to create less toxic dendrimers (Duncan and Izzo 2005).

Regarding *in vivo* studies Roberts et al. 1996, administered G3, G5 and G7 PAMAM dendrimers to groups of five male Swiss-Webster mice each, injecting 5×10^{-6} , 5×10^{-5} or 5×10^{-4} mmol/kg of each compound. The dendrimers were administered as a single dose or repeated once a week for 10 weeks and the observations were made by either 7 days or for a period of 6 months. In this study, there were no reported changes in behavior or weight loss in mice at 2 hours after administration, however, three animals died (injected with G7 PAMAM). In a multiple dose study, a degree of liver cell vacuolation was observed during histopathology that could be consistent with a lysosomal storage problem. Future studies are needed to corroborate these results (Duncan and Izzo 2005, Roberts et al. 1996).

In turn, three daily doses of PAMAM G3.5 administered to mice intraperitoneally at a daily dose of 95 mg/kg did not cause adverse changes on the weight of C57 mice, which carrying the B16F10 tumor line (Malik et al. 1999). When the melanin PEGylated dendrimers was injected into C3H male mice in a single dose of 2.56 g/kg intraperitoneal or 1.28 g/kg intravenous, non-toxicity or mortality was observed. In addition, after 24 h of intravenous administration and 48 h of intraperitoneal injection, no changes were observed in blood parameters (blood urea nitrogen levels or transaminases); however, a longer period of observation is needed to provide a more accurate response (Chen et al. 2004).

The applications of PAMAM dendrimers in drug delivery are limited given its inherent cytotoxicity, correlated with the generation of the dendrimer and the surface area of the dendrimer (Yellepeddi et al. 2009). Therefore, their toxicity could be reduced significantly by changes in its surface. Conjugation with PEG significantly decreased the cytotoxicity *in vitro* and *in vivo*, of G5 and G6 PAMAM dendrimers (Mishra et al. 2009, Qi et al. 2009).

PHARMACOKINETICS

Vehicles transporting polymeric drugs can be designated as nanoparticles that release encapsulated drugs via a surface diffusion or swelling, in a time or in a dependent-conditions manner. The release of the active agent may be constant over a long period of time, cyclical over a period of time, or can be triggered by environmental events or other external events (Kost and Langer 2001), such as changes in pH (Shmeeda et al. 2006, Yin et al. 2006) and temperature (Furgeson et al. 2006), like folate-conjugated liposomes which targeting the up-regulated folate receptors of cancer cells or Poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers that respond to temperature and pH. Other agents respond to the presence of an analyte such as glucose, composed of solid, particulate insulin, incorporated into an ethylene-vinyl acetate copolymer (EVAc) matrix (Brown et al. 1996). In general, polymer systems for controlled release should provide a release drug level in an optimum range over a long period of time compared to other methods of drug delivery. This increases the effectiveness of the drug and maximizes the patient's confidence, while improving the possibility of using highly toxic drugs, poorly soluble or relatively unstable. The first consideration in drug delivery is to achieve more effective therapies while eliminating the potential for overdose or inadequate dose (Alexis et al. 2008).

Another advantage of using controlled release systems include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient confidence. This is particularly relevant for cancer therapy, in which treatment effectiveness is directly related to the ability to kill cancer cells while affecting the fewest amount of healthy cells possible (Alexis et al. 2008).

As a general rule, for any carrier polymer to be used for parenteral applications is essential that this, be non-toxic, non-immunogenic, and preferably biodegradable. This should show a distribution in the body enabling the proper orientation to reach the desired tissue and away from sites that could be toxic (Duncan and Izzo 2005).

The need for biodegradable dendrimers emerged as a strategy to produce the desired high molecular weight carrier to reach a high accumulation and retention in tumor tissue while, in turn, allow the rapid and safe removal of the dendrimer fragments in the urine and avoid the non-specific toxicity. The biodegradable dendrimers are usually prepared by inclusion of ester groups in the polymer structure, which will be chemically hydrolyzed and/or enzymatically cleaved by esterases in physiological solutions (Lee et al. 2006, Morgan et al. 2006). Grinstaff, compared the rate of degradation of dendrimers G1 polyester [poly (glycerol-succinic) PGLSA] in the presence of acid, base and esterases, with polyester-amide dendrimers (G2) and polyester-ether (G3) to identify factors that control its kinetics degradation in physiological conditions (Grinstaff 2002). The results of this study showed that the polyester-ether dendrimers were the fastest to degrade due to the increased hydrolytic susceptibility compared to polyester and polyester-amide derivatives. Consequently, the following four factors seem to control the rate of degradation of the dendrimers: 1) the chemical nature of the bridges connecting the monomer units with ester bonds, being the most susceptible to hydrolysis compared with the amide and ether bonds, 2) hydrophobicity of monomer units, where more hydrophilic polymeric units (e.g., glycerol, lactic acid and succinic acid) result in rapid degradation compared to the hydrophobic monomers (e.g., phenylalanine and alkyl amines), 3) the fact that dendrimers with large sizes and molecular weights are degraded more slowly compared to smaller given the tight packing of its

surface, which effectively seals the hydrolysable groups, and 4) the susceptibility to cleavage of internal and external structure of the dendrimer, due to the inside hydrolysis leads to a faster degradation (Grinstaff 2002).

DRUGS CONJUGATION TO DENDRIMERS

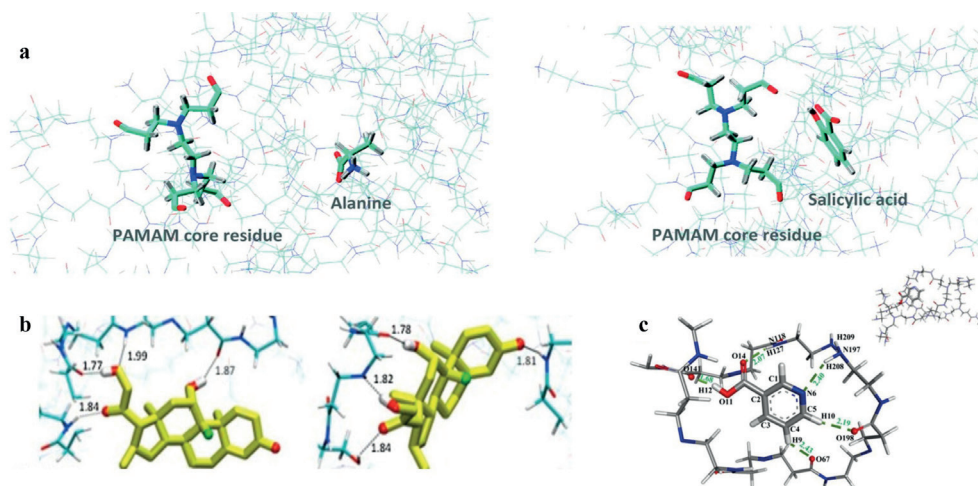
The drug delivery systems based on polymers are designed to improve pharmacokinetics and biodistribution of the drug and/or provide a controlled release kinetic in the target cell (Allen and Cullis 2004). The ideal dendrimer carrier should exhibit high water solubility, high drug carrying capacity, biodegradability, low toxicity, biodistribution characteristic, and favorable retention with appropriate specificity and bioavailability. In the release of drugs based on dendrimers, the drug is non-covalently encapsulated inside the dendrimer or covalently conjugated to form a macromolecular prodrug (Wolinsky and Grinstaff 2008).

DENDRIMER-DRUG COMPLEX

Buhleier et al. (1978) studied guest molecules trapped in branched polymers, representing an

early form of physical encapsulation of poorly soluble drug molecules in the spaces of dendrimers to improve its water solubility and controlled release profile (Bhadra et al. 2003, Morgan et al. 2006). The inclusion of hydrophobic molecules within dendrimers is typically achieved by simple mixing a polymer solution and drugs, where the hydrophobic drug associates with the nonpolar center via hydrophobic interactions (Morgan et al. 2003, Patri et al. 2005). As a result of the physical interface between the molecules and the dendrimer transporter (Figure 2), the release of encapsulated molecules in the aqueous environment is passively controlled by a range of non-covalent interactions including hydrophobic forces, hydrogen bonds, steric hindrance and Van der Waals electrostatic interactions (Medina and El-Sayed 2009, Mukherjee et al. 2015).

In this regard, camptothecin, a class of well-established anticancer drug with a very low solubility in water, was successfully encapsulated in a polyester dendrimer carboxylate terminated G4.5 to form a dendrimer-drug complex. All complexes showed much lower IC_{50} on cancer cells than free camptothecin (1.2 to 7.1 times the activity)



depending on the cell line used. The cellular uptake of the complex by MCF-7 cells was significantly higher (about 16 times) than those of the free drug. In addition, was observed a greater retention time of the drug inside the cell in the presence of dendrimers than in those in which the drug was free (Cheng and Xu 2008, Morgan et al. 2005).

Moreover, melanin-based dendrimers have been used to solubilize and reduce the toxicity of the anticancer drug methotrexate (MTX) and 6-mercaptopurine and reduce the toxicity of these drugs. In C3H mice, to which were administered subchronic doses of dendrimers with encapsulated drugs, were measured the levels of alanine amino transferase (ALT) to determine the hepatotoxicity, the ALT levels decreased by 27% for the dendrimer encapsulating MTX and 36% for the dendrimer encapsulating 6-mercaptopurine in comparison to animals receiving the drug alone (Neerman et al. 2004). The major drawback of these delivery

systems is the lack of controlled release kinetics of the drug, with most systems, releasing its load in the course of several hours. For this reason, dendrimers systems that encapsulate drugs could be better used via direct intratumoral injection (Wolinsky and Grinstaff 2008).

DENDRIMER-DRUG CONJUGATES

An alternative strategy for the use of dendrimers as carriers of anticancer drugs is making most of its well-defined multivalent structure in covalent bonds with the drug at its periphery. These examples are shown in Table I, the release of the drug can be adjusted by applying concepts of selective sites with degradable spacers between the drug and the dendrimer peripheral groups. Moreover, the burden of the drug to the dendrimer can be adjusted by varying the number of groups on the periphery of the dendrimer (Tekade et al. 2009).

TABLE I
Dendrimer - drug interactions on cancer therapy.

Dendrimer	Drug	Drug dendrimer interaction	Improved function	Ref.
Carboxylated PAMAM Dendrimer G3.5	Cisplatin	Covalent Conjugation	Improved loading efficiency, reduced cytotoxicity	(Nguyen et al. 2015)
Cyclic arginine-glycine-aspartic acid (RGD) peptide-conjugated generation 5 (G5) poly(amidoamine) dendrimers	Doxorubicin	Encapsulation	Form a water soluble complex, stable, and able to release the drug in a sustained manner	(He et al. 2015)
Omega-3 fatty acid [docosahexanoic acid (DHA)]-poly (amido)amine (PAMAM) dendrimer	Paclitaxel	Covalent Conjugation	Enhanced anticancer activity than Paclitaxel alone in various <i>in vitro</i> models of gastrointestinal cancer	(Dichwalkaret al. 2016)
Hyaluronic acid - amine terminated fourth generation poly(amidoamine) (PAMAM) dendrimer	3,4-Difluorobenzylidene Curcumin	Encapsulation	Target specificity and higher cellular uptake	(Kesharwaniet al. 2015)
6 kDa and 20 kDa Polyethylene glycol dendrimer	Paclitaxel	Covalent Conjugation	Prolonged residency and a sustained paclitaxel release, reducing local toxicity	(Luo et al. 2016)
56 kDa PEGylated polylysine dendrimer	Doxorubicin	Covalent Conjugation	Potential as inhalable drug delivery systems	(Kaminskas et al. 2014)

The utility of these degradable spacers was demonstrated by Greenfield et al. (1990) who use pH-sensitive bonds to adriamycin immunoconjugates. The conjugate was stable at physiological pH 7.4, but was found to undergo hydrolysis when was absorbed into the polymer by endocytosis and driven to subcellular organelles moderately acids such as endosomes and lysosomes. Keeping this model in mind, Ihre et al. (2002) reported the design and synthesis of a dendritic polyester system based on monomers 2,2-bis (hydroxymethyl) propanoic acid as a potential drug carrier. The drug doxorubicin was attached via pH-sensitive binding, demonstrating the feasibility of using these polyester dendritic structures to produce polymer-drug conjugates capable of releasing drugs to determined pH of cancer sites (Tekade et al. 2009).

In turn, hydroxyl-terminated G4 PAMAM in conjugation with paclitaxel through a union with succinic acid showed a significant increase in anticancer activity compared to free drug (10 times) (Khandare et al. 2006). Both conjugated, dendrimer-paclitaxel and PEG-paclitaxel, showed similar release profiles, across the membrane of cancer cells. The conjugate based on dendrimer had the highest anticancer activity than that based on PEG (250 times), suggesting that the dendrimer increases the levels of absorption of paclitaxel while PEG molecules decrease absorption by increasing the molecular weight of the drug. The dendrimers (carboxyl-terminated) conjugated with MTX via amide binding bridges was 3 to 8 times more potent than free MTX in MTX-sensitive cell lines (CCRF-CEM) and MTX-resistant (CEM/MTX), respectively (Gurdag et al. 2006). The conjugate was even 24 times more active than free MTX in other MTX-resistant cells (CHO) (Cheng and Xu 2008).

TARGETED CANCER THERAPY

The vasculature of tumors is highly heterogeneous, ranging from vascular necrosis areas to densely vascularized areas in order to sustain an adequate supply of oxygen and nutrients to the growing tumor. Tumor blood vessels have several abnormalities compared with normal blood vessels, including a high proportion of proliferating endothelial cells with an aberrant basement membrane, increased tortuosity of blood vessels and pericytes deficiency (Jain 2001). The microvasculature has also shown an increased permeability, which is regulated in part by abnormal secretion of vascular endothelial growth factor, bradykinin, nitric oxide, prostaglandins and matrix metalloproteinases. The transport of molecules can occur through the tumor microvasculature by opening inter-endothelial junctions and trans-endothelial channels (Alexis et al. 2008, Jain 2001).

Ideally, an anticancer drug should be first (after administration), able to achieve the desired tumor tissue through penetration of the barriers in the body with minimal loss of volume or activity in the bloodstream. Second, after reaching the tumor tissue, the drug should have the ability to selectively kill tumor cells without affecting normal cells, with a release mechanism controlled actively. In principle, the drug release of nanoparticles in tumor tissues can be addressed in two ways: passive and active (Misra et al. 2010).

PASSIVE DISTRIBUTION

The passive distribution refers to the accumulation of the drug or drug carrier system in the desired location given physico-chemical factors or pharmacological (Misra et al. 2010). Therapeutic macromolecules including drug delivery systems based on dendrimers, exploit the pathophysiological pattern of solid tumors, particularly its discrete vasculature to preferentially extravasate and accumulate in tumor tissue in a process known as

the effect of increased permeability and retention (EPR) (Maeda et al. 2000). The amount of the delivery system based on dendrimers, which accumulates in tumor tissue is influenced by the size, molecular weight and surface charge, which affects the residence time in the circulatory system, transport through the endothelial barrier, non-specific recognition and removal by the reticuloendothelial system (Haag and Kratz 2006). El-Sayed et al. (2002, 2003) study the effect of size, molecular weight, and surface charge on the permeability of PAMAM-NH₂ dendrimers (G0-G4) fluorescently labeled through the epithelial and endothelial barriers. Their data showed that the increase in the size/molecular weight of the dendrimer resulted in an exponential increase in the time of extravasation, through microvascular endothelium of the cremaster muscle in Syrian hamster (El-Sayed et al. 2001). A subsequent investigation by Kobayashi and Brechbiel (2005) studied the biodistribution of conjugates G2-NH₂ to G10-NH₂ functionalized with gadolinium, which were administered intravenously to normal mice. The results showed that the G2-G4 dendrimers functionalized with gadolinium was rapidly excreted in the urine after 3 minutes of intravenous injection while the G5 dendrimer and older generations showed a limited renal excretion due to its hydrodynamic volume. These studies show the influence of size and hydrodynamic volume of dendrimers in transport through the microvascular endothelium *in vivo* (Medina and El-Sayed 2009).

ACTIVE DISTRIBUTION

While the passive distribution of nanoparticles results in a preferential accumulation in tumors, an important non-specific binding of circulating nanoparticles also occurs in healthy organs. Therefore, a large area of research involves the functionalization of nanoparticles with specific fractions as monoclonal antibodies or ligands such

as vitamins, carbohydrate residues or peptides, which identify and bind to receptors either overexpressed in tumors or in the endothelium associated to them, maximizing the localization and accumulation in the tumor (Blanco et al. 2011, Brigger et al. 2002, Huynh et al. 2009).

When it builds a nanoparticle with tertiary structure (consisting of the drug and the fragment directed against the tumor), certain factors must be considered to create efficient delivery systems. First, antigen or receptor should be expressed exclusively in the tumor cell and not expressed in normal cells. Second, antigen or receptor should be expressed uniformly in all tumor cells. Finally, the antigen or receptor should not be released into the bloodstream. The internalization of the conjugate after binding to the tumor cell is an important criteria in selecting the appropriate ligand (Misra et al. 2010). For example, folate is a small organic molecule with high affinity to the folic acid receptor, highly overexpressed in certain tumors (100 to 300 times above endogenous levels) (Ross et al. 1994). PEGylated dendrimers functionalized with folate containing 5-fluorouracil (5-FU), showed to have a high accumulation in tumor of 20.1% and 10% of the injected dose at 8 and 24 hours, respectively, resulting in a significant reduction of tumor growth (approximately 40%) compared with non-functionalized controls after two injections (days 0 and 7) over 20 days (Blanco et al. 2011, Singh et al. 2008).

Other researchers have used different agents to reach the tumor including peptides to lead the drug delivery systems based on dendrimers to specific tumor receptors. Falciani et al. (2007) use neurotensin (NT) peptides to develop a dendrimer marked with NT carrying chlorine e6 (Cle6) and the chemotherapeutic agent MTX for various malignant tumors that express the receptor for NT, which includes carcinomas of colon, pancreatic, prostate and small cell lung. The treatment of mice carrying the tumor HT29 with conjugated

dendrimers-MTX-NT for 20 days showed a reduction in tumor size in approximately one third in comparison to mice that received saline or conjugate without MTX, indicating the therapeutic benefit of the active approach.

Another example is that of Hildgen, who developed an inclusion complex MTX-dendrimer polyether copolyester (PEPE) G2 for the treatment of brain tumors, which use as a ligand D-glucosamine disposed in the conjugate to link the transporters GLUT-1 highly expressed on the luminal side of endothelial cells of the blood-brain barrier and glioma cancer cells. *In vitro* studies showed that the conjugates MTX-PEPE exhibited 2 to 8 times greater accumulation in glioma cells, resulting in an effectiveness of 2 to 4.5 times greater than non-conjugated dendrimers (Dhanikula et al. 2008), other conjugated dendrimers used the tetrameric glycoprotein avidin to reach lectins differentially expressed on the surface of ovarian carcinoma cells (Xu et al. 2007). In turn, other constructs of PAMAM utilized the antibody J591 to reach the prostate-specific membrane antigen (PSMA), which is a glycoprotein expressed in all prostate cancer cells and the vasculature that supports it (Medina and El-Sayed 2009, Patri et al. 2004).

CONCLUSIONS

Nowadays, the ability to reach large numbers of drugs in specific tumor sites remains one of the greatest challenges in cancer therapy. In this regard, the use of dendrimers incorporating drugs as a complex, as polyester or melanin-based dendrimers carrying camptothecin or MTX have shown good results *in vitro* using breast cancer cell line, nevertheless, the lack of controlled release kinetics of the drugs continue being one of the main challenges of this method. For its part, the use of dendrimers conjugated with drugs have arisen as an alternative to the lack of control in the liberation of drugs, applying concepts of

selective sites with degradable spacers between the drug and the dendrimer peripheral groups, among them, dendritic polyester system based on monomers 2,2-bis (hydroxymethyl) propanoic acid attached to doxorubicin or hydroxyl-terminated G4 PAMAM in conjugation with paclitaxel through a union with succinic acid have shown great anticancer activity against ovarian carcinoma cells and acute lymphocytic leukemia cell line. However, no matter how the dendrimer transports the drug; an ideal dendrimer carrier should exhibit high water solubility, high drug carrying capacity, biodegradability, low toxicity, biodistribution characteristic, and favorable retention with appropriate specificity and bioavailability, characteristics that have not been fully covered by the current available dendrimers. Another important drawback is the heterogeneity of the cancer with cells that differ in their capacity of infiltration, their speed of growth, their capacity to form metastasis, their possibility of avoiding immune surveillance and other diverse characteristics, which makes it difficult to study the anticancer properties of the different dendrimers developed.

Despite the drawbacks mentioned above, the development and study of new dendrimers as drug-carriers continues to be an important tool in the cancer therapy as they can be functionalized with varied ligands to reach the tumor tissue through the different body barriers in the body with minimal loss of activity in the bloodstream, have the ability to selectively kill tumor cells without affecting the normal cells and most important with a release mechanism controlled actively.

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