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Review Article

Dendrimers and their applications

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Abstract

Dendrimers are symmetric molecules; their size is within the nanoscale. Its structure is usually homogeneous and monodisperse; they are composed of a nucleus and several layers ¹. The number of layers that the dendrimer has defines its generation. There are different types of dendrimers. The synthesis of these macromolecules is carried out following steps of growth and activation, and organic reactions are required to obtain their branched structure². Although size is essential, the determining factor of toxicity in dendrimers is the charge of the surface; it has been found that the higher generation dendrimers and the cationic ones are the most toxic compared to the lower generation anionics that evaluated at low concentrations did not show any toxicity ³. The dendrimers will favor the pharmacokinetics of a drug through the dendritic structure, the generation of the dendrimer to be used, the intramolecular interaction force between the adjacent functional groups in the dendrimer, the conditions of the environment such as pH, solvent polarity, strength ionic, saline concentration or presence of counterions, among others ⁴. Due to dendrimers' size and surface composition, the use of dendrimers in drug delivery has been increasingly studied. There are different interaction mechanisms between drugs in dendrimers, and these can be broadly divided into simple encapsulation, electrostatic interaction, and covalent bonds ⁵. The use of dendrimers in ocular administration has greatly impacted the complexity of this administration route. Gene therapy has also benefited from the emergence of these molecules as it facilitates targeted therapy.

Keywords: Dendrimers, Drug delivery, Gene therapy, Pharmacokinetic, Transport System

Introduction

Dendrimers are complex synthetic macromolecules, which are also known as cascade molecules. The term dendrimer arose from the derivation of the words "dendron" and "meros," which means "tree" and "parts," respectively, because dendrimers are synthetic polymers that have branched and defined three-dimensional architectures similar to a tree ^{6,7}.

In 1978 Voegtle et al. reported the first dendrimer-like compound; this was low-generation propylpropyleneimine (PPI). Later in 1980, dendrimers of higher generations were synthesized by Newkome, Tomalia, Frechet, and their colleagues; since then, multiple dendrimers with different structures and functions have been developed ⁶.

Dendrimers are made up of inner layers and outer layers; the outer layer is composed of functional groups that are useful for drug conjugation and specific groups that act on the target, while the inner layers are suitable for encapsulation of drug molecules with improved pharmacological efficacy, this reduces the toxicity of the drug and controls their release mechanisms ⁸.

A dendrimer is characterized by having three specific parts, which are: a central nucleus with two or more reactive groups, inner layers that are composed of repeated branching units that are covalently attached to the nucleus, these layers are called "generation " this is denoted as G_n where n can vary from 0 to 12 and by terminal functional groups on the outer surface ^{6,9}.

The dendrimers of smaller generations are asymmetric and with open conformations, while those of intermediate generations present semi-rigid structures with the capacity to transport other molecules inside and attached to their surface. Dendrimers of older generations are rigid molecules, so they cannot accept other particles in their interior. Furthermore, as the structures are larger, steric hindrance is generated by joining molecules on the surface. This is why middle generation dendrimers are preferred for transport ^{7,10}.

It is important to consider that the structure of the dendrimers can be precisely designed; during the synthesis, it is possible to control the branching and introduce changes in the terminal groups ⁹.

Many factors such as pH, the type of solvent, the saline effect, or the size can determine the physicochemical properties of dendrimers ¹¹. It has been discovered that the cytotoxicity associated with dendrimers depends in general on the generation, the number of groups found on the surface, and the nature of the terminal residues (cationic, anionic, and neutral), the highest cytotoxicity is associated with the dendrimers first-generation and cations. ⁹

The polyamidoamide dendrimer known as PAMAM have become very popular in the pharmaceutical industry since the chemical structure grows linearly in diameter and exponentially in the terminal groups so that the compounds of generation greater than G4-G6 will present intertwined branches, this allows it to generate geometrically closed nanostructures that allow them to be loaded with drugs ¹². This is an example that by strategically selecting the surface of the dendrimer, drug-dendrimer conjugates can be elaborated with defined structures that have specific properties; these can be endowed with selectivity and improve their solubility, among other characteristics, so they are a very promising vehicle in the pharmaceutical industry.

Advantages

Among the advantages offered by dendrimers can be found that they have reduced toxicity. This greater specificity results in the protection of healthy cells, tissues, and organs from the toxic side effects of the drug, they have an improved bioavailability, they have a prolonged half-life that results in the reduction of renal clearance and the protection of the incorporated drugs against premature enzymatic degradation and other elimination mechanisms, these can achieve a controlled or sustained release of the drug in order to maintain plasma levels and thus reduce the dosing frequency and dose. Furthermore, dendrimers can improve drug solubility and have the potential to be used as multifunctional excipients. ^{8,11,13}

It is essential to consider the advantages of dendrimers compared to liposomes since dendrimers can be covalently attached to drugs providing the stability that liposomes do not have as they do not have this type of union, which makes them challenging to stabilize. ¹²

Disadvantages

Among the disadvantages, it can be found that most dendrimers are not degradable in the physiological environment; this can lead to their accumulation within cells or tissues, causing severe side effects. ⁶

Types of Dendrimers

Polyester dendrimer

Generally, to obtain polymers with biodegradable bonds, one must work under rigorous synthetic conditions. However, this type of dendrimers presents a compromise between synthetic manipulation and biodegradability; after its discovery, it has been possible to synthesize polyester dendrimers that can be grouped into three categories: polyester dendrimers based on 2,2-bis (hydroxymethyl) propanoic acid (bis-HMPA) monomers, polyester dendrimers based on alternating monomers and the other types of polyester dendrimers ⁶. These dendrimers are characterized by being acidic, inexpensive, non-toxic, biodegradable, and non-immunogenic. They present a disadvantage in that they require multiple protection/deprotection reactions, which increases the risk of performing incomplete reactions and obtaining a defective product. ⁶

Polyacetal dendrimers

In 1992, polyacetal dendrimers with 2,4,8,10-tetraoxaspiro dendrons were synthesized using the transactivation technique of protection/deprotection sequences; in 2011, polyacetal dendrimers with PEO chains were synthesized, achieving the synthesis of seventh-generation dendrimers that included peripheral hydroxyls, which made it soluble in water. ⁶

PAMAM dendrimers

PAMAM (polyamidoamine) dendrimers have been widely used for drug delivery due to their biocompatibility, hydrophilicity, and lack of immunogenic effect. These generally have an ethylenediamine nucleus but can be synthesized with more hydrophobic interiors such as diaminobutane, diaminoethane, and diaminododecane, and in their branches, they have amine groups that can be used to load drugs, antibodies, enzymes, among others ^{8,14}. Low-generation PAMAMs have fewer surface primary amines and have a looser structure. In contrast, high-generation PAMAMs have many more surface primary amines resulting in more significant toxicity, including erythrocyte lysis due to the formation of nanoholes, erosion, and thinning of the membrane ^{15,16}. In addition, entire generations such as G1, G2, G3, and others end with -NH₂ groups and carry positive charges while half generations such as G0.5, G1.5, G2.5, among others, have -COOH carrying negative charges ¹⁵. In 1993 Haensler and Szoka discovered that PAMAM formed a complex with the DNA plasmid by electrostatic interaction. After this discovery, it was investigated on the different generations of PAMAM where it was found that the G1 cannot complex with nucleic acid due to the low density positively charged, G2s have a flat-elliptical shape, and they can form complexes with DNA but to a lesser extent than G4-G5. ¹⁴

Poly-L-lysine dendrimers

PLL (poly-L-lysine) dendrimers are synthesized by peptide bonds, and both the nucleus and the branches are based on the amino acid lysine; they have two primary amines generally modified to improve the therapeutic effect; these polymers are biocompatible, biodegradable, flexible, and soluble in water and are used to carry genes mainly. ^{8,14}

Propyleneimine dendrimers

PPI (propyleneimine) dendrimers were the first to be reported in 1978. These are mainly based on 1,4-diaminobutane (DAB), diaminoethane (DAE) nuclei with propyleneimine or ethylenediamine with branching units composed of propyleneimine monomers; in their inside, you can find tertiary amines of tris-propylene, the presence of alkyl groups in the branches makes them possess a more hydrophobic interior than the dendrimers of PAMAM. These dendrimers are used primarily for diagnosis. ^{7,13,19}

Synthesis of dendrimers

The organic synthesis of these polymers allows molecular parameters such as size, shape, interior and surface chemistry, flexibility, and topology almost perfectly.

Divergent growth method

It is the most used method. The construction of the dendrimer starts from the nucleus, and the addition of branches depends on the reactivity of the nucleus, which means that if the nucleus has two reactive ends, four branches are added. After having additional branches (a new generation), you must activate the exposed ends so that the next stage of branching can occur. These two steps, adding and activating, are repeated until the number of necessary generations is

reached; it is essential that the reaction is in order and is completed in its entirety to avoid defective branches. Among the advantages of this method, it can be found that at the end of the synthesis, the surface of the dendrimer can be modified with the desired functional groups, it is a fast synthesis, and it allows to prepare of large dendrimers. Among the disadvantages is the long necessary purification since the final product and the intermediate reagents have similar molecular weight, charges, and polarity. It is also necessary to use an excess of monomers in each of the reactions.^{7,15}

To synthesize PANAM dendrimers using this method, one begins with an ethylenediamine nucleus, with two branching ends of primary amine. To this structure, layers of methacrylate and ethylenediamine are added in successive reactions. These reactions generate carbon chains that have amide bonds inside and outside primary amines, to which new layers of methacrylate and ethylenediamine are added, which allows obtaining internal tertiary amines. Branches begin to be added from these tertiary amines inside the dendrimer, causing the dendrimer to grow generation after generation.⁷

Convergent growth method

It works oppositely to the model mentioned above. The synthesis begins with branching units called dendrons, which are synthesized from the outside to the inside, and this is why it is said that it occurs in the opposite way to divergent growth. However, the two techniques have in common that the branches are generated in two steps. In this case, the first of these steps is to activate the functional groups, and then the branches are added. When the dendrons are synthesized, they join the nucleus in the last stage to obtain the dendrimers. The advantage of this method is that it allows easier purification because it presents more significant differences between the final product and the intermediates; they also have greater monodispersity for low generations, present fewer defects in the branches, and present fewer defects in the branches errors in the synthesis. Since only correctly formed dendrons are selected to be attached to the nucleus. Among its disadvantages are that the yield is low, and it is difficult to obtain higher generations due to steric obstacles when the dendrons are connected to the nucleus.^{7,15}

Toxicity

Toxicity is defined as measuring the harmful effects on tissues, cells, and different body organs. For this reason, it is important to make different toxicity tests before their commercialization, to prioritize patients' security. To assure that dendrimers are biocompatible with cells, it is necessary the toxicological analysis. When analyzing dendrimers, their pharmacokinetic and distribution analysis are essential¹⁸. Over the years, scientists have investigated dendrimer's toxicity and discovered that the cationic ones, with higher generations, are the most toxic⁸.

According to a study applied to zebrafish embryo cells, made by Bodewein, L., Schmelter, F., et al., 2016, there is a strong influence of the size and generation factors in terms of its toxicity. Also, anionic dendrimers evaluated at low concentrations did not show any type of toxicity in the cells. However, the authors mentioned that at higher concentrations, this effect could not be assured¹⁹.

With their nanometric size, dendrimers can present problems derived from the capacity of interaction with cells and their components. For this reason, it is vital to study its toxicity to use them as therapeutic compounds transportation systems. It is said that its toxicity may be associated with: molecular weight, the charge density of the dendrimer, concentration of the dendrimer, exposure time, and type of exposed cell²⁰.

Due to the size of the dendrimer, which is generally found between 1 and 100 nm, they interact with cellular elements such as cell membrane, nucleus, and proteins, as well as with different ions, which can affect the hemoglobin's biological activity, as well as kidney function. While size is essential, the determining factor for dendrimer toxicity is the surface charge. The toxicity of dendrimers is influenced by pharmacokinetics and biodistribution, so biodistribution tests are of utmost importance to determine which are the target tissues²¹.

According to a study carried out by Hidalgo, S. 2018; in which a Janus type dendrimer was synthesized, it can be observed how the cytotoxicity of bedaquiline, which corresponds to a drug that under normal conditions presents high toxicity, decreases completely at any concentration of the dendrimer, for this reason, studies are suggested, since it is possible that when this change occurs in the cytotoxicity of a drug, it contributes to the creation of safer drugs, since one of the main problems of drugs, when they are synthesized, is their toxicity²².

Furthermore, in a study carried out by Diaz, C., Benitez, C., et al., 2018; where the characteristics of two synthesized PAMAM dendrimers are evaluated, when performing the cytotoxicity test in a human embryonic kidney, it is observed that cytotoxicity is observed at high concentrations while at low concentrations, which would typically be used for pharmaceutical treatments, cytotoxicity disappears in both dendrimers²³.

This can also be observed in a study by Thanh, V., Nguyen, T., Tran, T., Ngoc, U., Ho, M., et al., 2017; where two different PAMAM dendrimers are synthesized, in which a difference is observed in terms of their cytotoxicity, where one of these showed viability more significant than 60%, while in the other it decreased below 40%, however, when joining another dendrimer to each of these, it is observed how their cytotoxicity disappears, making them much more biocompatible²⁴.

Pharmacokinetic properties

Pharmacokinetics is defined as the pharmacology's branch that studies drug concentration changes, as well as its biotransformation products in biological fluids, tissues, or excretions, as a function of time through different models that describe, explain, and predict the effects of various absorption, distribution and elimination processes²⁵.

By using dendrimers as transporter systems of active compounds, their pharmacokinetic properties will be improved, since, with these, an improvement in their solubility, bioavailability, stability, a favoring in the accumulation of the drug at the site of action, in addition to a reduction in adverse reactions that a drug can cause¹¹.

As it is known, dendrimers will favor the pharmacokinetics of a drug; this improvement will depend not only on the use of these nanoparticles but also on the dendritic structure, the generation of the dendrimer to be used, the intramolecular interaction force between the functional groups adjacent conditions in the dendrimer, the medium conditions such as pH, solvent polarity, ionic strength, salt concentration or presence of counterions, among others⁴. It is important to mention that dendrimers are similar to proteins because they can get their native shape or be denatured according to their pH, ionic strength, or solvent polarity²⁶.

The dendritic structure comprises three main parts: a multivalent surface, an internal framework, and the nucleus or core; the latter varies according to the generation of the dendrimer¹⁴. In addition, it has been observed that hydrogen

bonds are formed between drugs and amino groups inside the dendrimer and the surface, so in the case of dendrimers with hydrophobic interiors, hydrophilic exteriors, these behave as micelles ²⁷. Also, when discussing dendritic structure, it is crucial to consider the surface's charge, because according to this, the permeability can be affected when administering drugs orally ²⁸.

On the other hand, as the generation of the dendrimer increases, in the same way, its size increases, so that the greater the generation of the dendrimer, the larger it will be; this, in turn, causes an increase in the internal cavities of the dendrimer, which increases the number of conjugations with the drug. However, it also becomes more rigid, making it challenging to release. Consequently, when using dendrimers as transporters, it is recommended to use dendrimers of the fourth generation or lower to control the particles ^{14,26}.

In addition, to improve the pharmacokinetic properties of drugs, linkers or spacers emerge, consisting of functional groups that will facilitate the cleavage of the complex and modulate the rate of drug release. According to the intramolecular force between the adjacent functional groups in the dendrimer and the drug to be transported, so will its solubility or selectivity characteristics. Among the existing linkers are amines, ester groups, disulfide bonds, among others ²⁹.

Another factor to take into account on excellent pharmacokinetics in the drug when using a dendrimer is the pH, which, being above 7, generates an increase in the size of the dendrimer, thus causing an increase in the generation of the dendrimer itself, which can favor the solubility and release of the drug ⁷.

Solvent's polarity must also be taken into account when using dendrimers as drug transporters since this, together with the purity of the solvent, affects the conformation of dendrimers due to the back-folding process of the terminal groups. In various studies, it was observed that polar dendrimers in non-polar solvents have more significant intra and intermolecular interactions, which produces a compact dendrimer interior and the aggregation of different dendrimers ³⁰.

Also, it is important to take into account the ionic force present between the dendrimer, the drug, and the medium, since the more significant the ionic force present between them, the release capacity will be reduced; however, if these ionic forces are controlled correctly, the degree of drug release can be favored ³¹.

Finally, it is crucial to take into account the saline concentration or the presence of counter ions, since when this concentration is low, the repulsion between the ionized groups of the dendrimer generates the extension of the dendritic structure, which in turn, produces more storage space of the drug within the dendrimer, thus favoring the amount of drug transported ³².

Dendrimers as a transport system

Due to the large surface area and the presence of functional groups in it, its high solubility in water, biocompatibility, and versatility, dendrimers have been used more and more as drug transporters. Due to their nanoscopic size, these polymers have opened a new dimension to the concept of controlled release. Another of the great advantages of these macromolecules being used as drug carriers is that they can transport both hydrophobic and hydrophilic molecules. ^{33,34}

Dendrimers can interact with molecules physically or chemically. When drugs are physically related to dendrimers, they do not undergo alterations. However, although these

interactions are easy to form, there is little control over their release. In addition, when these two structures are physically united, the drug: dendrimer ratio is usually low. Chemical interaction can take place in three different ways. The first of these is through a direct conjugation between the surface of the dendrimer and the drug molecule. They can also be attached using a linker, which is used when the drug does not have functional groups that can bind to the dendrimer or when this linker is necessary to modify solubility characteristics or the release profile. Another reason to use a different structure is to decrease the number of drug molecules near the surface of the dendrimer and thus obtain a more significant amount of drug bound to the dendrimer. Finally, the dendrimer can be made an integral part of the drug, which will be released in a specific tissue. ³⁵

Among the interactions that can occur is simple encapsulation, which occurs thanks to the spherical shape of the dendrimer and the internal cavity that it presents. Once inside this cavity, the drug can interact with specific groups in the dendrimer structure, such as oxygen or nitrogen atoms, allowing hydrogen bonds to form. However, no bond needs to occur as hydrogen bonds, and hydrophobic interactions can form in simple encapsulation, but physical encapsulation is also possible. This internal pocket has hydrophobic properties, making it possible to transport molecules that are not easily solubilized. ^{36,37}

Chemically dendrimers can transport drugs by electrostatically binding them with many functional groups found on the surface, such as amines or carboxyl groups. As an advantage when this interaction occurs, it can be seen that the solubility of hydrophobic molecules is increased; in addition to that, the drugs do not lose chemical or pharmacological integrity. Examples of these interactions have been seen with some NSAIDs that have carboxylic groups in their structure, such as ibuprofen or piroxicam, which form a stable complex with the dendrimer electrostatic interactions that are formed. ^{36,37}

Another way to join drug molecules to dendrimers to transport them is by joining these two structures through covalent bonds. In order to generate this union, it is necessary to have the presence of functional groups such as p-aminobenzoic acid or linkers of amides or esters. It should be mentioned that this form of transport is of special interest when it is wanted to have a controlled release of the drug, since this dendrimer-drug complex diffuses at a lower speed when compared to the free drug, also in targeted therapies since these complexes can be absorbed in specific tissues. A problem that can arise is that this complex is insoluble; however, this can be solved by joining small PEG chains. Some of the drugs that have been successfully linked to dendrimers include cisplatin, methotrexate, naproxen, and penicillin. ^{36,37}

When what is wanted is to carry drugs with hydrophilic characteristics, it is coupled to the dendrimer polyethylene glycol (PEG), creating unimolecular micelles that can, on this surface of PEG, solubilize hydrophilic molecules. In contrast, inside the dendrimer, it can carry hydrophobic molecules. ³⁶

Ocular Administration

When a drug is used through the ophthalmic route, it comes into contact with the ocular surface, which is made up of the precorneal area (lipid characteristics) that includes the cornea, the conjunctiva, and a layer of mucins that are responsible for regulating the pH ocular; and the post corneal area where the retina is located. (38,39) When administered, drugs are absorbed in the first 30 minutes to exert local action; however, these can also pass from the tear duct (which is part of the conjunctiva) to the nasal cavity or to the

lymphatic system, where they are absorbed and pass into the circulation causing systemic effects.^{38,40}

Any drug administered to exert action on the back of the eye must pass through the precorneal area, as mentioned above, and the corneal and post corneal area, which is why only 3-5% reaches the intraocular tissues. One of the reasons why the bioavailability of drugs administered by the ocular route is so low is because the epithelium of the cornea has its cells closely linked together, generating a selective barrier, in addition to the stroma; Another structure of the cornea has very hydrophilic characteristics, making the transport of lipophilic molecules difficult. Lastly, we can mention the enzymes present in the ocular cavity that cause degradation of the administered drugs.³⁸

The magnitude of the effects produced is determined by the amount of drug administered and by the bioavailability of the drugs, the latter being conditioned by the active drug, the anatomophysiological characteristics of the ocular apparatus, and the pharmaceutical form, for which reason the use of dendrimers in this route of administration has been studied.⁴¹

Therefore, the main challenge in the administration of drugs at the ocular level is to increase the bioavailability and prolong the residence time of the drug with the surfaces of the eye. This is why different polymers have been created to transport drugs, however, if these are synthesized with very small sizes, they are quickly drained through the tear duct, and if they are large, they cause eye irritation and are eliminated by tears. In addition, they tend to infiltrate the lacrimal gland, causing a decrease in the flow of tears, which causes blurred vision.³⁷

Although dendrimers are polymers, they have different characteristics that make them good candidates for transporting drugs through the ophthalmic route since they can dissolve hydrophobic drugs in the ophthalmic cavity and retain and maintain a controlled release of active ingredients.³⁷

Some dendrimers have a cationic charge that is important for ocular administration since the charges they present interact with the mucins of the corneal epithelium, which contains sialic groups that, at physiological pH, are charged negatively. Due to this electrostatic interaction, dendrimers function as mucoadhesive polymers, increasing the contact time of the active drug and the ocular surface.⁴²

On the other hand, there is also an electrostatic interaction with the proteins of the epithelial intercellular junctions, causing a temporary reorganization of these proteins, which increases paracellular permeability. All of this promotes the entry of drugs into the cornea.⁴²

Gene therapy

Gene therapy is known as the set of techniques that allow conveying DNA or RNA sequences to a target cell to modulate the expression of certain altered proteins, which means that cellular deficiencies expressed in the phenotype are corrected, thus reversing any biological disorder that occurs.^{37,43} This mobilization of exogenous genes occurs through vectors; these facilitate entry and improve their bioavailability. There are two types of vectors, viral and non-viral, within which are dendrimers.¹⁴

Due to the high immunogenic response that viral vectors induce, non-viral vectors have been increasingly studied, which are easily manufactured with highly defined structures, generate less immune response, and, in addition, can be modified to direct their transport.²⁰

The most suitable dendrimers for transporting DNA are those containing positively charged groups on their surface, such as

PANAMs or PPIs, since due to this positive surface charge, they can condense the DNA, forming a stable complex; which protects the oligonucleotide from degradation and which is also able to cross cell membranes and release its contents into the target cells, since, as mentioned above, dendrimers can be modified to improve their specificity for the cells of interest.²⁰

The dendrimer-DNA complex is formed by electrostatic-hydrophobic interactions. They occur between the amines of the dendrimer and the phosphate groups of the nucleic acid; These positive and negative charges will be neutralized, and as a consequence, on some occasions, it goes from an extended structure to a compact one. The nature of this complex will not depend solely on the stoichiometry and concentration of the groups that form the interactions, but the solvent in which the complex is formed and its properties, such as pH and salt concentration, will also be important.^{20,45}

Therefore, different conditions will define the affinity of the complex that is formed. One of these is the size of the dendrimer. The larger this is, the greater the number of terminal amines the molecule will present and, therefore, the greater amount of positive charges, generating more possibilities of contact with the DNA chains. To guarantee that the amines present in the structure are protonated, they must be in an environment with a pH close to 7 (physiological pH). If the dendrimer were in a solution with a higher pH, the amines would be in their neutral form, so they would be unable to form bonds with nucleic acids. Whereas, if the pH of the solution is very low, the chloride ions will bind to the positive charges both on the surface and inside the dendrimer, competing and weakening the dendrimer-DNA bond.²⁰

Another critical factor in the complex formation is the ratio of charges present, which are described by a factor called R. This value relates to the number of positive charges provided by the amines of the dendrimer and the negative charges of the phosphate groups of the chains of DNA. When R is less than 1, the formed complex exhibits a low affinity, increasing the possibility of dissociation. On the other hand, if R is greater than 1, the positive charges exceed the amount of the negative charges, producing coiling of the DNA chains on the dendrimer, overcoming the energy and entropic barriers, forming a more stable complex; however, it must be taken into account that if R is extensive, the complex is so stable that it may not dissociate when it reaches its target.²⁰

The concentration of counterions and water molecules also determines the binding affinity of the dendrimer-DNA complex. Before the interaction occurs, both the dendrimer and the DNA are neutralized by Cl⁻ and Na⁺ and solvated by water molecules. When the complex begins to form, these ions come out, and by concentrating in the solvent hinders the binding and affinity of the complex since the contacts and the degree of bending of the DNA chains are reduced. Lastly, the length of the DNA chain can also influence the flexibility or stiffness, which is present when complexing with the dendrimer²⁰.

Once the complex is formed, both the dendrimer and the DNA will undergo conformational changes, which will be determined by the size of the dendrimer. Small dendrimers will undergo a more significant deformation than the larger ones. This happens because the dendrimer expands its surface to increase the contact points with the nucleic acid; in this way, the smaller dendrimer loses its spherical shape and passes to a more extended and asymmetric conformation. On the other hand, DNA shows a more remarkable change when it complexes with larger dendrimers. When the DNA chains face a small dendrimer, there is little charge ratio, which results in a stretching of the DNA and adsorbs to the dendrimer's surface without it suffering twists, resulting in a weak bond.

While joining a larger dendrimer and a DNA chain, it is compacted to a greater extent and supports twisting, bending, and shortening due to the neutralization; all this results in a curling of the DNA chain on the dendrimer. When dendrimers are large, what occurs is penetration of DNA into the dendrimer, which later hinders its release ²⁰.

DNA transfection from the dendrimer to the cell begins when the dendrimer-DNA complex is endocytosed by the cell, thanks to interactions between the positive charge of the dendrimer and the negative charge on the cell membrane. An endosome is formed from this endocytosis, a vesicle with the dendrimer-DNA complex that separates it from the cytosol. Although several endocytosis mechanisms have been described, two stand out, the one mediated by clathrin and the one mediated by caveola ^{20,46}.

In the clathrin-mediated endocytosis process, there is the formation of an invagination of the cell membrane together with the polymerization of the clathrin protein, which forms a polygonal network that gives structure and covers the dendrimer-DNA complex; forming an endosome. Clathrin polymerization occurs when three heavy chains and three light clathrin chains are associated, forming what is known as triskelions; when these structures are assembled, the complex is covered. Assembling proteins such as the adapter protein AP-2 participate in this process; in addition, the dynamic GTPase is responsible for separating the vesicle from the membrane. This endocytic process ends with the association with lysosomes in a pathway of acidification of the complex and degradation of endocytosed particles ⁴⁶.

On the other hand, the endocytosis of the dendrimer-DNA complex mediated by caveolae occurs through an invagination of the cell membrane in lipid rafts known as caveolae. These structures correspond to lipid microdomains that are formed around the caveolin-1 protein. This membrane protein that does not expose sites towards the extracellular zone has a site that binds cholesterol and sphingolipids in its N-terminal region, creating a zone enriched in these lipids. When this pathway forms the endosome, it has been seen that these drift goes towards the Golgi apparatus and/or the endoplasmic reticulum, in a route where there would be no acidification or degradation, although it has also been described that in some instances, they end up associated with lysosomes ⁴⁶.

The release of the contents of the endosome sometimes occurs by a rupture due to a "proton sponge" effect. This effect occurs because the complex can buffer the pH since the amines of the dendrimer can capture protons, but there is a flow of chloride into the endosome. Due to this increase in ions, there is an attraction and entry of water into the endosome, causing the breakdown and exit of the genetic material, which goes to the nucleus ²⁰.

Biodistribution

Dendritic compounds considered for biomedical applications by general rules must have low toxicity profiles, a time in circulation long enough for therapeutic efficacy to be achieved. They must be easily eliminated from the body to avoid possible unacceptable accumulation in the long term ⁴⁷.

Among the most important factors to consider in the elimination or excretion of dendrimers, either by renal or hepatic clearance, it is necessary to:

- The size of the dendrimer must be between 10-100nm to have optimal properties for in vivo administration since sizes less than 10nm are extravasated into tissues and undergo rapid renal clearance, and sizes greater than 100nm are opsonized and eliminated from the bloodstream by the macrophages using the

reticuloendothelial system ⁴⁸. Dendrimers with higher G will be cleared by the kidneys more slowly, so they will have longer retention in the blood ⁴⁹.

- The density of the surface charge, since it has been shown that cationic dendrimers are easily eliminated from the circulation and accumulate significantly in the liver, anionic dendrimers remain longer in the blood and accumulate in the liver to a lesser extent than the cationic, hydrophilic dendrimers remain circulating for a long time since they prevent rapid elimination ¹¹.
- Naturaleza hidrófila/hidrófoba del núcleo del dendrímero ya que se ha observado que aquellos con un interior más hidrofóbico se eliminan más rápidamente de la circulación sanguínea acumulándose 2 veces más en el hígado que los que poseen un centro hidrofílico, esto se debe tomar en cuenta ya que hace más difícil de predecir la toxicidad a largo plazo. ^{11,50}

Conclusions

Among the different dendrimers, polyester dendrimers are acidic, non-toxic, low cost, biodegradable and non-immunogenic; however, they require multiple protection/deprotection reactions, as do they require multiple protection/deprotection reactions polyacetal dendrimers, of which there are seventh-generation water-soluble dendrimers. PAMAM dendrimers are widely used in drug delivery due to their biocompatibility, hydrophilicity, and non-immunogenic; however, the generation used must be considered since they can be toxic or interfere with DNA. Finally, there are PLL dendrimers used mainly to carry genes and PPIs used for diagnostics. All these different types can be synthesized using the convergent or divergent growth method.

Thanks to the different properties of dendrimers, they are becoming increasingly attractive for use as drug delivery media. Gene therapy has benefited from these molecules since they make it possible to direct the therapy. On the other hand, dendrimers are also used in ocular administration since they help the drug to remain in contact with the surface for longer and promote its entry into the cornea.

Dendrimers are nanoparticles that are of great importance at the pharmaceutical level since due to their reduced toxicity, greater specificity, improved bioavailability, prolonged half-life, and their ability to bind to drugs covalently, they have demonstrated improved solubility and potential for use as multifunctional excipients, always taking into account that they are not degradable in a physiological environment so that they can present essential side effects. They can also be toxic depending on their generation, charge, and concentration. Undoubtedly, these are molecules that are here to stay and innovate the types of formulations that exist in the market, and the pharmaceutical industry has been able to exploit them little by little to obtain better results every day in the health of people and fewer adverse effects to improve the quality of life of patients.

Conflicts of interest

The authors declare that no conflict of interest exists.

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