DENDRIMERS – AS NEW PROMISING TOOLS IN THE MANAGEMENT OF MULTI-DRUG RESISTANT BACTERIAL INFECTIONS

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ABSTRACT

Antibiotic resistance represents a global threat. In recent years, an alarming increase in cases of infections with multidrug resistant bacteria has been reported. In this context, it is necessary to find new therapeutic approaches. Dendrimers are new artificial macromolecules with a highly branched three dimensional structure. Recently, many researchers have focused on investigating the possible applications of dendrimers in different medical fields. Dendrimers proved to be useful in the treatment of bacterial infections. They exhibit antibacterial effects or act as carriers for antibiotics. Thus, dendrimers may be a promising weapon against multidrug resistant bacteria, but several peculiarities of these molecules, like their cytotoxicity, biodisponibility, half life etc. should also be considered.

Keywords: dendrimers, multiresistant bacteria, antimicrobial activity.

REZUMAT

Rezistența la antibiotice reprezintă o amenințare la nivel global. În ultimii ani, s-a înregistrat o creștere alarmantă a cazurilor de infecții cu bacterii MDR (multidrug resistant). În acest context, este necesară dezvoltarea unor noi agenți terapeutici. Dendrimerii sunt o nouă clasă de macromolecule de sinteză, cu o structură tridimensională foarte ramificată. Recent, numeroși cercetători și-au îndreptat atenția spre investigarea posibilelor aplicații ale dendrimerilor în diferite domenii medicale. Dendrimerii s-au dovedit a fi utili în tratamentul infecțiilor bacteriene. Aceștia pot avea un efect antibacterian direct sau pot acționa drept cărăuși pentru antibiotice și chimioterapice. Astfel, pot reprezenta o armă promițătoare împotriva agenților microbieni rezistenți, dar trebuie avute în vedere o serie de proprietăți ale acestora, cum sunt: citotoxicitatea, biodisponibilitatea, timpul de înjumătățire etc.

Cuvinte-cheie: dendrimeri, bacterii multirezistente, activitate antimicrobiană.

1. INTRODUCTION

Dendrimers are polymers that have similar properties to proteins, but are less compacted than a protein, having highly branched structures [1, 2]. They were first described in 1978 by Vogtle *et al.* as "cascade molecules" [3]. Dendrimer comes from the Greek word "*dendron*" which means tree. Dendrimers have a wide applicability in various medical fields [4]. However, dendrimers are very expensive molecules that are not easily obtained, given the high degree of branching and their perfect structure [5]. There are many studies available on this topic. Dendrimers are able to sequester various compounds such as proteins, nucleic acids, bile acids, ions and, for example, can be useful, in the treatment of renal chronic disease or haemochromatosis [6, 7]. Dendrimers can also be employed in gene therapy or as antitumor agents [8, 9]. They can be used in the treatment of infectious diseases (bacterial, viral and parasitic infections) and represent a promising tool in the management of multidrug resistant bacterial infections [10, 11].

Bacteria are microorganisms capable of adapting to different conditions. Thus, long ex-

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posure to antibiotics led to the occurrence of resistance. Various mechanisms of resistance to antibiotics have been described. Antibiotic resistance represents an important public health issue and a pressing problem for the development of new therapeutic molecules [12]. "Every year, approximately 4 million patients contract an infection in the European Union and about 37,000 die as a direct consequence of the infection" [13]. The number of new antibiotics is small in relation to the rapid rhythm of emergence of resistant bacterial strains [14]. In this context, it is necessary to find solutions to use the pre-existing antibiotics or to introduce other antimicrobial molecules [15].

The structure of dendrimers varies and, depending on the functional groups, they can be used in different medical fields. For example, dendrimers with polylysine groups seem to be useful as antibacterial and antiviral agents, and can also be used to develop vaccines [16].

2. Structure, synthesis and characteristics

Dendrimers have a perfect three-dimensional geometry, which distinguishes them from linear polymers. Dendrimers are among the smallest nanosystems. The structure of dendrimers is based on polyamines (PPI dendrimer) or a combination of polyamides and amines (PAMAM dendrimer); some of them present subunits, e.g. aryl-ether group, which confer them hydrophobicity [1]. Newer dendrimers include carbohydrates in the structure of their nucleus or contain elements such as silicon or phosphorus [17].

From a structural point of view, the dendrimers include three main parts: the multivalent surface with an increased number of reactive loci, the dendrons (the branches) and the core for dendron attachment. The external structure is made up of covalent bonds which confer it a lower flexibility [4, 18]. The core comprises one or two atoms having at least two functional chemical groups that allow the branches to bind. The branches include repetitive units with at least one junction of ramification. The repetitive units are organized in a geometric progression, resulting concentric layers called generations. Every ramification starting from the inner part towards the external part of the dendrimer is a generation. High generation dendrimers are larger and more branched. The surface groups are terminal functional groups responsible for the effects of the dendrimers. The solubility of dendrimers is dependent on the type of the surface groups. Dendrimers with terminal hydrophilic groups are soluble in polar solvents and those with terminal hydrophobic groups in non-polar solvents [19]. Regarding their shape and size, insulin, cytochrome and hemoglobin have approximately the same shape and size as low generation PAMAM dendrimers (G3, G4 or G5). Higher generation dendrimers PAMAM (G7) resemble lipid bilayer membranes [20].

Two types of dendrimer synthesis, convergent and divergent have been described. Dendrimers can be synthesized divergently starting from the core to the periphery or convergently in the opposite direction [21].

In the divergent method, the core reacts with monomeric structures containing a reactive group and two dormant groups, giving birth to the first generation. These molecules react with other monomers and thus form several generations, layer by layer. Using the divergent method, it is difficult to form dendrimers with more than 6 generations. The divergent method presents the disadvantage of a higher rate of imperfections in dendrimer structure because of incomplete reactions of the terminal groups [22]. One of the main advantages is that the surface functional groups can be easily modified. In addition, it is a fast method which allows the production of a large quantity of dendrimers. Despite the aforementioned disadvantages, the advantages of the method make it the most used one. Currently, the divergent method is preferred; therefore, this assortment is the most commercialized by industrial companies. [16].

The convergent method is based on the synthesis of dendrimer beginning from the surface groups and progressing to the dendrimer core. After synthesizing sufficiently large branches, they are attached to the nucleus [10, 22]. As an advantage of convergent method compared to the divergent method, the final

product is easier to purify and the structural defects occur less frequently. Another advantage of convergent synthesis is that each dendron may be different and many functional groups with various roles may be incorporated into the same dendrimer [5, 23]. During the convergent synthesis the molecular weight can be controlled [24].

Dendrimers have to meet certain characteristics for safe use. First of all, they should be non-toxic, with the mention that higher generation dendrimers exhibit enhanced cytotoxicity. From a structural point of view, they have to exhibit the ability to pass cell membranes. Immunogenicity is an important aspect which should also be considered. Recent studies on PAMAM dendrimers have revealed that they are molecules with a low immunogenicity [1]. In addition, it seems that the functionalization of the amino-terminated PAMAM dendrimers with polyethylene glycol groups decreases the immunogenicity [25]. Another important concern is that dendrimers have to target specific structures in order to achieve the expected effects. An important feature of dendrimers compared to linear proteins is the uniform dispersity, which has been highlighted by several methods such as HPLC, mass spectrophotometry and gel electrophoresis. Monodispersity gives the investigators the chance to use a tool for well-defined scalable sizes [24].

Dendrimers can play two main roles; they can act as drugs themselves or can be carriers for different agents [26]. Both hydrophobic and hydrophilic elements can be incorporated into a dendrimer. The hydrophobic agents will be encapsulated inside the dendrimer and the hydrophilic agents will be attached to the multivalent functional groups of the dendrimer [23, 27]. PAMAM is the most used dendrimer for the release of antimicrobial agents [28].

In terms of pharmacokinetics, the properties of dendrimers such as the molecular weight and nature of surface functional groups are among the most important. For example, the polycationic dendrimer PAMAM showed a rapid clearance from blood circulation after intravenous or intraperitoneal administration and accumulated mainly in the liver or kidney. The PAMAM dendrimer that was modified in order to be negatively charged persisted longer in the blood stream. Research has shown that dendrimers with a molecular weight greater than 40 kDa persist longer in circulation than those with a lower weight. In addition, it seems that the persistence in blood circulation is also influenced by the degree of branching; the highly branched dendrimers persist longer (11). Some researchers have synthesized dendrimers that cross the hematoencephalic barrier. G5 PAMAM conjugated to 5-fluorouracil was used to obtain a better bioavailability [5].

3. Designing dendrimers

Designing a dendrimer is a complex process aiming at obtaining a compound best adapted to a certain application. Dendrimers used for *in vivo* drug delivery must meet several criteria, such as: being non-toxic, having a good bioavailability in respect of time of circulation and access to specific target structures etc. Their structure and electrical charge has to best fit the molecule that we need to attach and then deliver for therapeutical purposes.

Attaching a drug to the suitable dendrimer may increase its aqueous solubility, its circulation half-life, improve its bioavailability, biodegradability and excretion time etc [19].

Despite the large spectrum of possibilities to adapt the dendrimer – antimicrobial compound characteristics, it is not easy to find the best variant resulting from combining all needed requirements. Therefore, several researchers in the field investigated the physicochemical properties of dendrimers by using computer simulations. Computer programs have been optimized by using a large amount of data, in order to compare predictions based theoretical calculations and experimental results obtained by chemical analysis [29].

Several types of dendrimers have been designed. Peptide dendrimers are made up of a core consisting of di, tetra or octavalent lysine coupled with octa-peptides [30]. It has been shown that peptide dendrimers are directly effective on Gram-positive bacteria (e.g., *Staphylococcus aureus, Enterococcus faecalis*, etc.) and Gram-negative bacteria (*Escherichia*

coli, Pseudomonas aeruginosa, etc.) [31, 32]. Peptide dendrimers are resistant to proteases and less cytotoxic. Another type of dendrimer is PAMAM dendrimer that can be combined with different particles such as silicone by attaching a mannose derivative. They can be used to prevent the formation of biofilm on various surfaces, allowing the formation of biofilm by non-pathogenic *E. coli* that binds to mannose and prevents the formation of biofilm by pathogenic bacteria [33].

PAMAM dendrimers are the most used dendrimers, being active on *P. aeruginosa* and *S. aureus* [34]. A higher dendrimer concentration is required for *S. aureus*. This can be explained by the thicker wall of Gram-positive bacteria. PAMAM dendrimers are the first dendrimers that were synthesized, thoroughly analyzed and marketed, most studies being based on this type of dendrimers [20]. Carbosilane dendrimers, which are among the newer types of dendrimers [35] contain quaternary ammonium terminal units. They exhibit amphiphilic properties and water solubility only in the presence of dimethyl sulfoxide. A better activity against *S. aureus* than *E. coli* was revealed. Multilingual dendrimers are dendrimers which exhibit several copies of the same functional group on their surface [2].

4. Cytotoxicity

Cationic dendrimers exhibit the ability to interact with the lipid bilayer from cell membranes leading to an increased permeability and decreased cell membrane integrity. Classical representatives of this class are PAMAM, PPI and polylysine dendrimers. Among these, it was observed that PAMAM dendrimers cause concentration-related and generation-dependent cytotoxic effects. In addition, increased membrane affinity of dendrimers is associated with increased cytotoxicity and it has also been shown that there is a positive correlation between citotoxicity and the amount of positive charges. The latter can be reduced by acetylation or incresed by lipidation [36].

With regard to toxicity, studies have shown that if a long blood circulation time is required, the dendrimer must be negatively charged or uncharged, which reduces the non-specific interactions with hepatic tissue, the site where the dendrimer accumulates [11]. It has been concluded that cationic dendrimers are more cytotoxic than anionic or PEGylated dendrimers [37]. *In vitro* studies have been conducted to assess the toxicity of various modified or native dendrimers on different cell lines [38, 39].

However, few researchers have evaluated the toxicity of dendrimers *in vivo*. According to the first *in vivo* toxicology study, a dose of 10 mg/kg appears to be non-toxic in the case of PAMAM dendrimers (G1-G5) when the agent is injected into mice and all generations tested were found to be non-immunogenic [38, 39]. Recent studies have been conducted on PAMAM dendrimers with amino-type groups showing that intravenous administration was lethal in mice and caused a disseminated intravascular coagulation status [40].

By using flow cytometry and microscopic analysis it was demonstrated that G7 PAMAM dendrimers labelled with fluorescein isothiocyanate might induce perturbations of the platelet function, while PAMAM dendrimers with hydroxyl terminal groups and anionic dendrimers with carboxyl terminal groups did not alter platelet morphology or their function [40]. These recent results are consistent with the observations from previous studies and reveal that anionic dendrimers are less toxic as compared to cationic dendrimers [37]. The toxicity profile of G5 PPI dendrimer has also been investigated. Functionalization with t-Boc, mannose and tuftsin resulted in significant reduction in PPI toxicity. In fact, this process leads to the covering of primary amino groups responsible for the positive charge and consequently for an increased toxicity. It was concluded that the functionalization of dendrimers contributes to both the reduction in their toxicity and increase in biocompatibility [41].

5. Dendrimer interactions with drug molecules

The dendrimer-drug interaction can be achieved in three main ways, namely, drug encapsulation, covalent conjugation and electrostatic interaction. The activity

of dendrimers is dependent on generation, hydrophilicity, concentration and functional terminal groups. The first studies performed the non-covalent encapsulation of some molecules; DNA was incorporated and gene delivery was accomplished [42].

a. *Simple encapsulation*

This process involves the incorporation of the drug into the dendrimer and the drug is sequestered into the cavities of the dendrimer. These internal cavities typically have hydrophobic properties, making them suitable for interacting with less soluble drugs. In addition, in the cavities, there is oxygen or hydrogen, ions that can interact with the drug by forming hydrogen bonds [5, 43].

b. Electrostatic interaction

The presence of multiple functional groups on the surface of dendrimers contributes to the increased solubility of some drugs through an electrostatic interaction. An electrostatic interaction involves the formation of complexes between ionizable drugs with different ionizable functional groups of the dendrimers [43, 44].

c. Covalent conjugation

Covalent bonds with various drugs can be established through the surface functional groups of dendrimers. The drug is covalently bound and the release of the drug is made through a chemical or enzymatic cleavage [1].

6. Dendrimers and dendrimer conjugates as antimicrobial agents

Dendrimers find applicability in the treatment of multi-resistant bacterial infections. Cationic dendrimers are positively charged, and the bacteria membrane is negatively charged. There are interactions that lead to an increased permeability of the bacterial membrane [7]. Cationic dendrimers may be toxic to mammalian cells, but structural changes have been made resulting in decreased toxicity. Anionic dendrimers have lower toxicity. Antimicrobial agents encapsulated inside the dendrimer may target the wall or membrane of the bacteria. In addition, the conversion of terminal groups to ammonium salts may result in the formation of dendrimers with a biocidal effect [26].

PAMAM dendrimers may present different terminal groups, those with terminal amino groups having the highest antibacterial activity [45].

Dendrimers can be very useful to improve drug pharmacokinetics. Thus, for example, the oral bioavailability of drugs with low water-solubility may be increased. Surface groups have an important role in this process [46]. Hydrosoluble functional groups may be added resulting in water-soluble dendrimers. Thus, for example PAMAM dendrimers can increase the water-solubility and antimicrobial activity of sulfonamides. The study by Winnicka *et al*. showed that PAMAM dendrimers can increase the solubility of erythromycin, the solubility being dose-dependent; however the antibacterial activity of erythromycin was not modified [45].

Fluoroquinolones are well absorbed in the digestive tract, but the most common side effects are on the digestive tract. The study by Cheng *et al*. showed that encapsulation of nadifloxacin and prulifloxacin in PAMAM dendrimers leads to a similar effect to the administration of the pure drugs themselves [47]. Metallodendrimers, dendrimers that have the ability to incorporate metal atoms, have also been studied. For example, antibacterial activity against *S. aureus* was tested using silver nanoparticles with favorable results. In addition, dendrimers can bind toxins and neutralize them [26].

Another study was conducted on the use of neutral fourth generation (G4) PAMAM dendrimers as a drug-delivery agent for azithromycin in *Chlamydia trachomatis* genital infections. After successfully conjugating the 2 molecules, the conjugate (D-AZ) was found to be significantly better than the free drug in preventing productive infections in the cells and better in reducing the number and size of chlamydial inclusions [48].

Another study, where the bound antibiotic was sulfamethoxazole (SMZ) and the dendrimer was a third generation (G3) PAMAM, showed that dendrimers could increase the anti-bacterial activity of SMZ (a 4- or 8-fold increase in the anti-bacterial activity of SMZ

in dendrimer solution compared to pure SMZ dissolved in dimethylsulfoxide or 0.01 M NaOH solution). The *in vitro* release behavior and anti-bacterial activity studies indicated that PAMAM dendrimers might be considered as potential drug carriers of sulfonamides with a sustained release behavior under suitable conditions [49].

The drug carrier potential of fifth generation (G5) PAMAM dendrimers was also observed in a study where the antibiotic being carried was Vancomycin. The study proposed the conjugate of dendrimer–based vacomycin as a potential new compound for targeting Gram-positive bacteria [50].

An interesting study was also performed using an antimicrobial polypeptide (AMP) bound to a third generation PAMAM dendrimer (G3KL). The study aimed at testing the antimicrobial capabilities of the compound against various multidrug-resistant strains of *Acinetobacter baumannii* and *P. aeruginosa* and comparing it to various other standard antibiotics. The study concluded that G3KL is a promising molecule even against extensively drug resistant *A. baumannii* and *P. aeruginosa* [51].

Another recent study on the antibacterial activity of dendrimers involved a seventh generation (G7) PAMAM dendrimer. No other molecule was bound to the dendrimer, but instead its own antibacterial activity was investigated using various strains of *P. aeruginosa* (n = 15), *E. coli* (n = 15), *A. baumannii* (n = 15), *Shigella dysenteriae* (n = 15), *Klebsiella pneumoniae* (n = 10), *Proteus mirabilis* (n = 15), *S. aureus* (n = 15) and *Bacillus subtilis* (n = 10). The study concluded that the PAMAM-G7 dendrimer could be used as a novel antibacterial agent [52].

We should take into accont that the interaction between dendrimer and various molecules is influenced by pH level. Moreover, the ability of dendrimers to adapt their structure to nanoscopic spaces is also influenced by pH level [53, 54].

7. CONCLUSION

Dendrimers are highly branched polymers that can interact with various molecules, finding applicability in various medical fields. They have a perfect three-dimensional structure with the surface functional groups being responsible for their properties. Recent studies have revealed their role as antimicrobial agents. Dendrimers may represent a new approach for infections with multi-drug resistant bacteria. However further studies on efficacy, cytotoxicity, pharmacokinetic/ pharmacodynamic properties etc. are required.

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