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Research Article

Structure-Activity Relationship of Anionic and Cationic Polyamidoamine (PAMAM) Dendrimers against *Staphylococcus aureus*

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Dendrimers are potent synergists, carriers, and delivery molecules for natural biological products and pharmaceuticals. *Staphylococcus aureus* (*S. aureus*) infection is causing serious diseases in humans and animals. Given the recorded antibacterial and antiviral activity of terminal-charged PAMAM dendrimers, the relation between dendrimer charge type and generation is to be established against *S. aureus*. Three types of polyanionic dendrimers comprising terminal groups sodium carboxylate (generations 1.5, 2.5, 3.5, and 4.5), hydroxyl (generations 2, 3, 4, and 5), and succinamic acid (generations 2, 3, 4, and 5) and polycationic dendrimers containing primary amine (generations 2, 3, 4, and 5) were in antibacterial assays to determine their zone of inhibition and antibacterial activity. Cationic dendrimers were more potent than anionic dendrimers. The largest inhibition was shown by G(5)-128NH2 followed by G(4)-64NH2 primary amine dendrimers. Carboxylate, hydroxyl, and succinamic acid dendrimers showed weaker effects. Owing to their antibacterial actions, the addition of dendrimers to antibiotic preparations may increase their efficacy by their intrinsic and bacterial action by damaging the bacterial membranes as well as their usage in drug delivery.

1. Introduction

Dendrimers are considered potent synergists for natural plant products and the delivery of pharmaceuticals. Considerable improvement of ursolic acid water solubility was accomplished by its encapsulation in dendrimer nanoparticles [1]. PAMAM dendrimers have shown absorption-increasing effects on liquiritin, as well as spleen and liver protection, detoxification, and other activities, which had not been widely employed in clinical application due to their poor absorption, first-pass effect, and limited bioavailability [2]. PAMAM dendrimers were also found to be an effective adsorbent for extracting flavonoids from plant extracts [3]. The dendrimers are composed of a set of substructures called dendrons, which comprises tree-like branches of chemical constituents. The overall structure of dendrimers has three main components, a core moiety, branching units, and surface groups [4]. Dendrimers span a nanosize scale, and their size could be comparable to globular proteins. For illustration, G4 and G5 PAMAM dendrimers have a diameter of 4 nm and 5 nm, which is equal to cytochrome c and hemoglobin, respectively. Therefore, dendrimers can be suitable biomimetic agents with improved stability and biological activity, compared with proteins [5]. More interestingly, the production of glycodendrimers overcame the poor affinity of simple saccharides due to the formation of strong multivalent binding and disruption of bacterial lectins. Based on this strategy, glycodendrimers were synthesized and effectively prevented infection of several bacteria such as *Escherichia coli* (*E. coli*) [6], *Vibrio cholera* (*V. cholera*) [7], *Pseudomonas aeruginosa* (*P. aeruginosa*) [8], and *Haemophilus influenza* (*H. influenza*). In general conclusion, the power of dendrimers arises from their multivalency. Multivalent substitutions in dendrimers are a potent

replacement of single molecules due to multiplex and extensive interaction with biological macromolecules and higher binding affinity.

Dendrimers showed unique intrinsic antimicrobial properties including antiviral activity [9]. The predicted antiviral actions of dendrimers comprise influenza virus [10], human immunodeficiency virus [11], measles virus [12], dengue virus [13], and respiratory syncytial virus [14]. Dendrimers have recently been used as a platform for the treatment and detection of SARS-CoV-2 through their use as nanocarriers or nanodrugs in the treatment of COVID-19 [15]. In a phase 2a therapeutic trial, patients with severe COVID-19 received OP-101, a hydroxyl-polyamidoamine dendrimer–N-acetyl cysteine combination that targets active macrophages. Op-101 was well tolerated and reduced the probability of death or mechanical ventilation at 30 and 60 days following treatment compared to a control group [16]. Treatment with OP-101 resulted in a decrease in serum levels of proinflammatory and neurological indicators (neurofilament light chain and glial fibrillary acidic protein). It is possible that OP-101 could be used to treat COVID-19's systemic hyperinflammation.

S. aureus has undergone a series of unique strain emergence events during the current antibiotic era, several of which have included the acquisition of antibiotic resistance. There is a great deal of concern about the prevalence of methicillin-resistant *S. aureus* (MRSA) in healthcare facilities and the general public [17]. This study was carried out to interpolate the effect of dendrimer size and the variable terminal charge on the Gram-positive bacteria, *S. aureus*. The structure-activity relationship is to be established. Dendrimers were specifically used for their wide applications as nanocarriers for drug delivery and their antimicrobial properties.

2. Materials and Methods

2.1. Dendrimers. All dendrimers were synthesized by Dendritech Inc. (http://www.dendritech.com/pamam.html, Midland, Michigan, USA). The dendrimer set includes three different polyanionic and one polycationic dendrimers. The polyanionic dendrimer sets comprise 1.5-5 generations and three different terminal functional groups, hydroxyl, carboxyl, and succinamic acid terminated PAMAMs. The polycationic dendrimers comprise primary amine terminal groups (Figure 1).

2.2. Bacterial Strain and Culture Media. S. aureus (ATCC29213) was used to evaluate the antibacterial activity. The stock culture was stored at -80°C in 50% glycerol. For bacterial propagation, 100 μl from stock cultures was plated on brain heart infusion agar (Oxoid, UK) for 24 h at 37°C.

2.3. Antibacterial Activity. The antibacterial activity of the 16 dendrimers was preliminarily evaluated by the disc diffusion test as per standards defined by (CLSI 2014). Bacterial cells from overnight growth on brain heart infusion agar were adjusted to be equivalent to a 0.5 McFarland standard which corresponded to approximately 108 CFU/ml. An aliquot of 100 μl from the adjusted cells was inoculated onto Müeller-Hinton Agar (Fluka, Biochemika). For the preparation of dried filter paper discs, Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized by autoclaving. The sterilized discs were impregnated with 10 μl of dendrimer stock solutions to obtain a final concentration of 1 mM/ml and placed onto inoculated agar plates and incubated at 37°C for 24 h. Each concentration was tested in triplicate. 100% dimethyl sulphoxide (DMSO) (10 μl) was used as the negative control, and cefotaxime 10 μg/ml was used as a positive control. For the estimation of MIC, twofold dilution of the dendrimers was carried out in a fresh sterile LB medium. Serial dilution was adjusted to deliver final concentrations of 1-256 μg/ml.

3. Results and Discussion

In this study, we used anionic and cationic dendrimers in different terminal charges and generations to evaluate their antimicrobial activity against *S. aureus*. Overall, the cationic dendrimers gave a larger zone of inhibition, compared with the anionic dendrimers (Figure 2). The distribution of the inhibitory zone of the various dendrimers against bacteria is shown in Table 1. The highest growth inhibition of *S. aureus* was produced by G(5)-128NH2 followed by G(4)-64NH2 and G(3)-32NH2. In this set of primary amines, the larger the PAMAM generation the higher the obtained inhibition zones. Small zones of inhibition were observed with sodium carboxylate dendrimers, in which smaller generations were more effective than larger generations. In contrast to primary amine dendrimers, the smaller generation carboxylate dendrimers produced higher inhibition zones. For instance, G(1.5)-16COONa produced the largest inhibition zone, which decreased gradually by using G2.5 and G3.5 dendrimers. The hydroxyl dendrimers showed a weak effect and the lowest inhibition zone among all dendrimers. The succinamic acid dendrimers showed more or less similar profiles across their G2-G5 dendrimers. For further analysis of the efficacy of the strongest dendrimer, the MIC50 of G(5)-128NH2 was estimated.

In a recent report, we used the same set of dendrimers to determine its antiviral activity against MERS-CoV [18]. In this study, the strongest antiviral effect was delivered by G(1.5)-16COONa (40.5% inhibition), followed by G(5)-128SA (39.77% inhibition). The differences in the antiviral and antibacterial actions might be attributed to the differences in the outer charges of bacterial walls and viral envelopes.

The principal antibacterial mode of action of cationic dendrimers is to be like the pore-forming defensins. Dendrons (part of dendrimers) can assemble into highly membrane-active hydrophobic segments and porous supramolecular columns. Additionally, dendrimers' actions can be best described as a quaternary ammonium compound. It adsorbs to the highly anionic heavily negatively charged bacterial outer layer, followed by diffusion into cell membranes, binding, and disruption of cell membrane followed by cell lysis. The effect of dendrimers with their multivalent

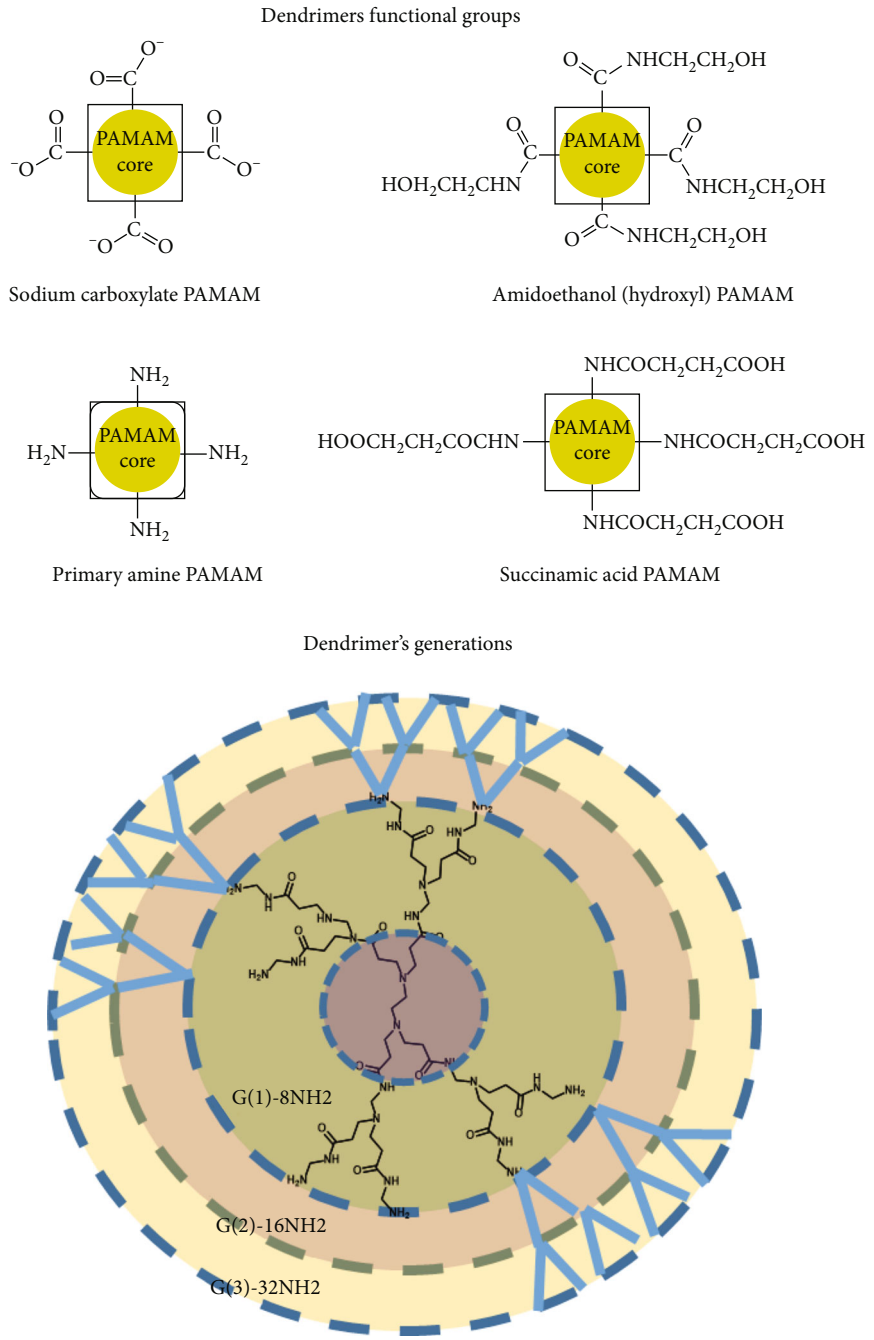

Figure 1: The structure of the PAMAM dendrimers used in this study. Terminal groups used in this study were sodium carboxylate, primary amine, hydroxyl, and succinamic acid.

composition is highly potent compared with single cationic molecules [19–21].

More recently, electrostatic-based modification of PAMAM dendrimers enhanced their biofilm penetration and their antimicrobial activity against *S. aureus [22]*. Owing to its antibacterial actions, PAMAM dendrimers have been used as an alternative to traditional methods for eliminating harmful bacteria and organic and inorganic pollutants [23]. PAMAM-antibiotic nanofibers for covering wounds were also used for controlling infections [24]. Taking into account the abundance of negative charges on the bacterial outer layers, it becomes extremely susceptible to dendrimers at concentrations greatly lower than the expected toxic concentrations of mammalian cells. The provided structure-activity relationship in this study can support the use of PAMAM dendrimers in nonmaterial-based chemotherapy.

4. Conclusions

S. aureus is an excellent test organism for establishing the charge type-generation relationship of PAMAM dendrimers as well as their antibacterial and antiviral properties.

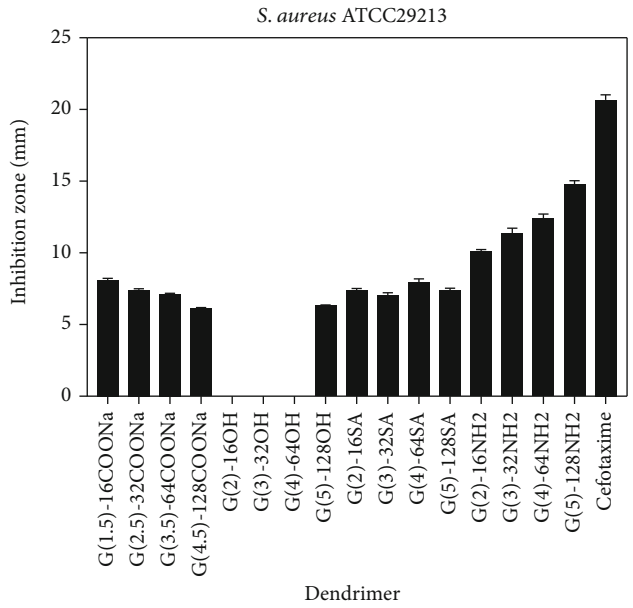

Figure 2: The inhibition zone diameter produced by different anionic and cationic PAMAM dendrimers against *S. aureus*. The value represents the mean and SD from three different experiments.

Table 1: The inhibition zone diameter produced by different anionic and cationic PAMAM dendrimers against *S. aureus*. The value represents the mean and SD from three different experiments.

Compounds	Identity	*S. aureus* ATCC29213
C1	G(1.5)-16COONa	8 ± 0.2
C2	G(2.5)-32COONa	7.3 ± 0.3
C3	G(3.5)-64COONa	7 ± 0.1
C4	G(4.5)-128COONa	7 ± 0.1
C5	G(2)-16OH	6 ± 0.1
C6	G(3)-32OH	0
C7	G(4)-64OH	0
C8	G(5)-128OH	6.2 ± 0.1
C9	G(2)-16SA	7.3 ± 0.3
C10	G(3)-32SA	6.9 ± 0.3
C11	G(4)-64SA	7.8 ± 0.4
C12	G(5)-128SA	7.3 ± 0.2
C13	G(2)-16NH2	10 ± 0.3
C14	G(3)-32NH2	11.2 ± 0.5
C15	G(4)-64NH2	12.3 ± 0.4
C16	G(5)-128NH2	14.6 ± 0.4
Cefotaxime		22.5 ± 0.6

Polyanionic dendrimers with terminal sodium carboxylate (generations 1, 2, 3, and 4.5) and hydroxyl (generations 2, 3, and 5) groups, as well as polycationic dendrimers with primary amine (generations 2, 3, and 5) groups, were tested in antibacterial assays to determine their inhibitory zone and antibacterial activity. Cationic dendrimers were more potent than anionic dendrimers. The most effective inhibitors were discovered to be G(5)-128NH2 and G(4)-64NH2 primary amine dendrimers. Dendrimers containing carboxylate, hydroxyl, and succinamic acid showed a lower effect. Dendrimers, which have antibacterial qualities, can be added to antibiotics to increase their efficacy by breaking bacterial membranes and more effectively delivering the medications.

Data Availability

All data are within the manuscript.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

MK and YA prepared the original draft and revised the manuscript. MK acquired funding. All authors have read and agreed to the published version of the manuscript.

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