Summary

Bacterial resistance to antibiotics is currently one of the most serious challenges in modern medicine, leading to increased morbidity and mortality worldwide. Gram-negative bacteria are a particular concern, as their outer membrane constitutes an effective barrier that limits antibiotic penetration. Due to the limited efficacy of conventional therapies, alternative therapeutic strategies based on antimicrobial proteins and nanomaterials, including dendrimers, are gaining increasing importance. Proteins such as phage-derived endolysins degrade the peptidoglycan layer of the bacterial cell wall, whereas dendrimers can increase outer- membrane permeability, thereby supporting the activity of endolysins against Gram- negative bacteria. The aim of this study was to determine the effect of cationic carbosilane dendrimers containing imidazole rings (Dend), silver ions (DendAg), as well as pyridyl rings with silver ions (DendCh, DendAgCh) on the susceptibility of *Pseudomonas aeruginosa* PAO1, and to evaluate their ability to enhance the activity of the CHAP domain of an endolysin derived from bacteriophage Φ812. The *P. aeruginosa* strain was selected due to its high level of resistance resulting from mutations, enzymatic modifications, and its ability to form biofilms.

In this work, it was demonstrated that cationic dendrimers inhibit bacterial growth and induce outer membrane permeabilization, which facilitates endolysin penetration to the peptidoglycan layer and its degradation. However, the degree of permeabilization depended on the dendrimer type. In addition, the enzymatic activity of the endolysin toward peptidoglycan increased in the presence of dendrimers. Thermodynamic analysis of the interactions between dendrimers and the endolysin showed that these compounds associate via electrostatic interactions in an exothermic process (DendAg, DendCh, DendAgCh) at low temperatures and an endothermic process (Dend) at higher temperatures. The synergistic activity of dendrimers and the endolysin against bacteria was also evaluated – three of the four dendrimers exhibited synergistic effects, which justified excluding Dend from further studies. The morphology and size of dendrimers and their complexes with the endolysin were determined using transmission electron microscopy (TEM) and dynamic light scattering (DLS). Due to the hydrophobic nature of the molecules, a tendency to form aggregates with sizes of 350–800 nm was observed. In the next stage, the production of reactive oxygen species (ROS) was analyzed. Dendrimers containing silver ions increased ROS levels. Bacterial viability in the presence of dendrimers and the endolysin was assessed by fluorescence microscopy – in all cases, a decrease in the number of live cells and an increase

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in dead cells were observed. Observations by fluorescence microscopy, SEM, and TEM confirmed numerous membrane disruptions in the form of pores as well as cell wall degradation, indicating the lytic activity of the studied systems. The effects of dendrimers and their complexes with the endolysin on the complex structure of bacterial biofilms were also assessed. First, the ability of dendrimers to inhibit biofilm formation at increasing concentrations was examined, and subsequently the same experiments were performed in the presence of the endolysin. It was shown that endolysin alone enhanced the antibiofilm activity of dendrimers, whereas dendrimers used individually did not produce this effect. Microscopic analysis revealed a decrease in the number of live cells and an increase in dead bacteria within the biofilm, indicating disruption of biofilm structure and bactericidal activity of the dendrimer–endolysin complexes. The next stage involved evaluating the effects of dendrimers and the endolysin on biofilms formed on porcine skin, used as an *ex vivo* model of skin infection. Fluorescence microscopy and SEM analyses showed that the most pronounced antibiofilm activity was observed for DendAg and DendAgCh, and to a lesser extent for DendCh. An increased number of dead cells, numerous bacterial cell wall disruptions, and changes in the biofilm matrix structure were observed. Histomorphological analysis of non-infected and *P. aeruginosa*-infected skin after application of dendrimers and the endolysin was also performed. In non-infected skin, dendrimers affected epidermal structures to varying degrees, leading to atrophy, hypertrophy, or hyperplasia and local disruption of continuity between layers. In infected skin, the epidermis was completely degraded by bacteria, with partial degradation of the dermis, which prevented assessment of a potential therapeutic effect. Finally, the cytotoxicity of dendrimers and the effect of endolysin on mitigating this toxicity were evaluated in VH10 fibroblast cultures. Cytotoxicity increased with dendrimer concentration, whereas the presence of endolysin slightly reduced this effect.

In summary, cationic carbosilane dendrimers, depending on the type of modification, enhance endolysin activity and exhibit antibacterial effects against *Pseudomonas aeruginosa* PAO1 through mechanisms involving membrane permeabilization, peptidoglycan degradation, and the generation of reactive oxygen species. These compounds also contribute to biofilm reduction under *in vitro* and *ex vivo* conditions. However, they display a certain degree of cytotoxicity, the extent of which depends on concentration and the type of dendrimer surface modification.

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Lail Vlaudine