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Structure–activity relationships in aminosterol antibiotics: The effect of stereochemistry at the 7-OH group

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ABSTRACT

Squalamine and three aminosterol analogs have been shown to inhibit bacterial cell growth and induce lysis of large unilamellar phospholipid vesicles. The analogs differ in the identity of the polyamine attached at C3 of the sterol, and the stereochemistry of a hydroxyl substituent at C7. Analogs with a tetraammonium spermine polyamine are somewhat more active than analogs with a shorter trisammonium spermidine polyamine, and analogs with an axial (α) hydroxyl substituent at C7 are more active than analogs with the corresponding equatorial (β) hydroxyl group. There is some variability noted; the 7β-OH spermine analog is the most active compound against *Escherichia coli*, but the least effective against *Pseudomonas aeruginosa*. Lytic activity correlates well with antimicrobial activity of the compounds, but the lytic activity varies with the phospholipid composition of the vesicles.

The ongoing discovery of antibiotic-resistant bacterial strains, coupled with the limited development of new antimicrobial agents, has caused alarm among both physicians and research scientists.[1,2] Most commonly prescribed antibiotics work by one of four general mechanisms. Antibiotics in the penicillin and cephalosporin classes work by inhibiting bacterial cell wall synthesis. Tetracylines and chloramphenicol inhibit bacterial protein synthesis, while fluoroquinolones and nitroimidazoles inhibit nucleic acid synthesis. Finally, sulfonamides inhibit bacterial growth by blocking folic acid biosynthesis.[3] Many bacterial strains have developed resistance to one or more of these different types of antibiotic action. Thus, the best method available for combatting antibiotic-resistant bacterial infections is by aggressive multidrug therapy.[4–6]

The development of new biocidal agents will help to minimize the acquisition of microbial infections generated from public contact. Biocides are chemical agents capable of killing living organisms. Most biocides interact with the outer membranes of the organisms they kill, but the mechanisms causing cell death are varied and not completely understood.[3] Many biocides are cationic compounds, suggesting an interaction with the anionic plasma membranes of pathogens.[7] The common structural elements in cationic biocides are polar cationic groups attached to a large hydrophobic entity. The cationic groups are often ammonium groups, but the molecular architecture and organization can vary dramatically while maintaining strong biocidal activity.[7]

Our laboratory has a long-standing interest in squalamine (3β-spermidine-7α-hydroxy-5α-cholestan-24*R*-yl sulfate; see Fig. 1 for structure) and related aminosterols. Squalamine exhibits broad spectrum antimicrobial and antifungal activity, with little to no effect on mammalian cells.[8] Our laboratory has synthesized a large number of squalamine analogs, and all exhibit at least modest antimicrobial activity.[9,10] We have demonstrated that squalamine's antimicrobial activity is related to membrane lysis. Squalamine preferentially interacts with anionic lipids in liposomes, which results in the formation of membrane defects leading to vesicle lysis.[11] Until now, we have only studied vesicle lysis caused by squalamine, and have not measured the lytic activity of other aminosterols.

In this study, we compare the lytic activities of squalamine and three analogs, which vary in the number of cationic groups within the polyamine substituent at the sterol C3 carbon and the stereochemistry of the 7-hydroxyl substituent. All four compounds are strikingly soluble in water, and all possess significant antimicrobial activity.[9] From this work, we confirm some correlation between the antimicrobial activity of aminosterols with their ability to lyse phospholipid membranes, and have discovered that the orientation of the C7 hydroxyl influences both the antimicrobial and lytic activities of the aminosterol.

Abbreviations: DOPG, dioleoylphosphatidylglycerol; DOPC, dioleoylphosphatidylcholine; MIC, minimum inhibitory concentration; LUV, large unilamellar vesicle.

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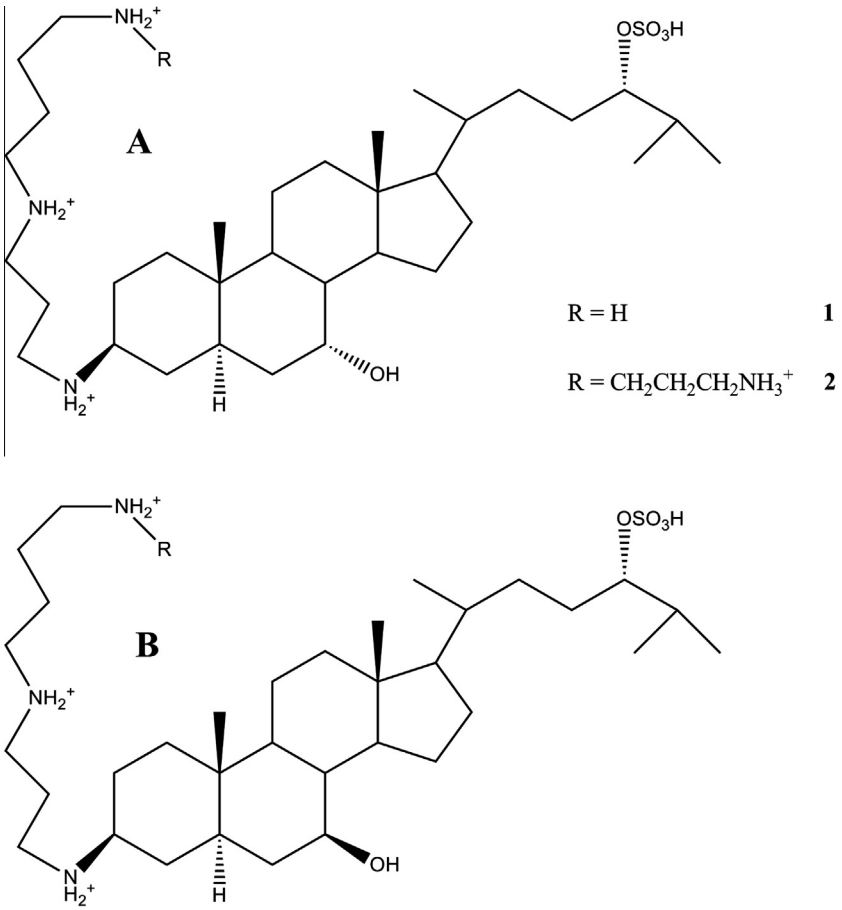

Figure 1. Structures of the aminosterols used in this study: (A) naturally occurring aminosterols with a C7 axial hydroxyl substituent, with either a spermidine (squalamine; R = H) or spermine (MSI 1436; R = propylamine) polyamine attached at C3; (B) aminosterol analogs with a C7 equatorial hydroxyl substituent.

Squalamine (3β-spermidine-7α-hydroxy-5α-cholestan-24*R*-yl sulfate) as its dilactate salt and its 3β-spermine analog (also referred to as trodusquemine or MSI 1436[12]) as its HCl salt were the kind gifts of Dr. Michael Zasloff at Georgetown University. In addition to being a potent biocidal compound, trodusquemine has been shown to suppress appetite and cause significant weight loss in obese mice.[13,14] 3β-Spermidine-7β-hydroxy-5α-cholestan-24*R*-yl sulfate and 3β-spermine-7β-hydroxy-5α-cholestan-24*R*-yl sulfate were synthesized as their trifluoroacetate salts as previously described.[9] The structures of the four compounds are provided in Figure 1. For membrane lysis and antimicrobial assays, stock solutions of the above compounds were prepared at a concentration of 1 mg/mL in distilled water and stored at −20 °C until use. When needed, lower concentration solutions were prepared by dilution of freshly thawed stock solutions into distilled water.

The ability of the four aminosterols (Fig. 1) to cause the leakage of the fluorescent dye calcein from phospholipid LUVs prepared from dioleoylphosphatidylglycerol (DOPG), dioleoylphosphatidylcholine (DOPC), and a 1:1 DOPC:DOPG mixture was measured. Vesicles were prepared by membrane extrusion[11,15], and their phospholipid concentration determined by total phosphate assay.[16] Figure 2 describes the leakage of calcein from DOPG LUVs after a one minute exposure to each of the aminosterols, plotted as a function of the aminosterol/phospholipid molar ratio.[17] The two aminosterols with the spermine polyamine substituent were equally effective in causing dye leakage from unilamellar vesicles. Squalamine, with its shorter spermidine polyamine substituent, was slightly less effective in causing calcein leakage than the spermine compounds. The fourth compound, 7β-hydroxy, 3β-spermidine, was much less effective in causing vesicle leakage, requiring much higher aminosterol/phospholipid molar ratios to generate the leakage observed using the other three agents.

The leakage of calcein generated by aminosterols from LUVs prepared from a 1:1 molar ratio of DOPC:DOPG (1:1) is described in Figure 3. In this system, the 7α-hydroxy, 3β-spermine and 7α-hydroxy, 3β-spermidine aminosterols were the most effective in causing leakage from LUVs. The analog with the longer polyamine is slightly more effective in causing leakage than the analog with the shorter spermidine polyamine. However, both analogs with a 7β hydroxyl substituent were less effective in causing vesicle leakage. The 7β-hydroxy, 3β-spermine analog was somewhat more effective in causing leakage than the 7β-hydroxy, 3β-spermidine analog. This is not surprising, given the longer, more positively charged substituent on the spermine analog and the previously determined interaction between the positively charged amines and the negatively charged phospholipid headgroups.[11]

In comparing Figures 2 and 3, a shift in the effectiveness of the different aminosterols is noted. When vesicles prepared entirely from the anionic lipid DOPG are tested, the two analogs with the longer spermine polyamine are most effective. However, when the vesicles are prepared from a mixture of DOPG/DOPC, the 7α-hydroxy analogs are preferred over the 7β analogs independent of the length of the polyamine substituent. The reason for this shift is not clear, and awaits additional studies with analogs with shorter and/or less charged amine substituents.

Figure 4 examines the ability of the aminosterols to generate calcein leakage from LUVs prepared entirely from the zwitterionic phospholipid DOPC. The leakage behavior of the two spermine ana-