Sulfonium-based liposome-encapsulated antibiotics deliver a synergistic antibacterial activity

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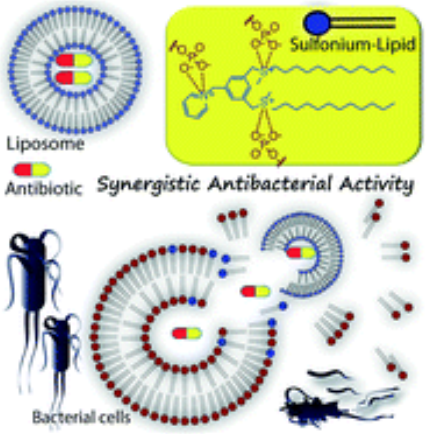
Abstract

The devastating antibacterial infections, coupled with their antibiotic resistance abilities, emphasize the need for effective antibacterial therapeutics. In this prospect, liposomal delivery systems have been employed in improving the efficacy of the antibacterial agents. The liposome-based antibiotics enhance the therapeutic index of the new or existing antibiotics and reduce their adverse effects. The current study describes the development of sulfonium-based antibacterial lipids that demonstrate the delivery of existing antibiotics. The presence of cationic sulfonium moieties and inherent membrane targeting abilities of the lipids could be beneficial in reducing the antibiotic resistance abilities of the bacteria and delivering the antibiotics to remove the infectious pathogens electively. The transmission electron microscopic images and dynamic light scattering analyses revealed the liposome formation abilities of the sulfonium-based amphiphilic compounds in the aqueous medium. The effectiveness of the compounds was tested against the gram-negative and gram-positive bacterial strains. The viability of the bacterial cells was remarkably reduced in the presence of the compounds. The sulfonium-based compounds with pyridinium moiety and long hydrocarbon chains showed the most potent antibacterial activities among the tested compounds. Mechanistic studies revealed the membrane-targeted bactericidal activities of the compounds. The potent compound also showed tetracycline and amoxicillin encapsulation and sustained release profiles in the physiologically relevant medium. The tetracycline and amoxicillin encapsulated lipid showed much higher antibacterial activities in comparison with the free antibiotics at similar concentrations, emphasizing the usefulness of the synergistic effect of sulfonium-based lipid and the antibiotics signifying that the sulfonium lipid penetrated the bacterial membrane and increased the cellular uptake of the antibiotics. Hence, the sulfonium-based lipid exemplifies a promising framework for assimilating various warheads and provides a potent antibacterial material.



Keyword- Antimicrobial activity, Synergistic effect

The anticancer drug delivery and moderate antimicrobial activities of the sulfonium lipids motivated us to synthesize a new series of sulfonium-based compounds. The key features in designing the compounds were the installation of cationic (pyridinium) or anionic (sulfonic acid) headgroup and variation of alkyl chain length in addition to the sulfonium moieties (Scheme 1). The variation in alkyl chain length would allow us to investigate the role of hydrophobicity and antimicrobial activity. We envisage that the hydrophobicity of dialkyl chain lengths could allow the compounds to self-aggregate in aqueous medium, which could be useful in encapsulating the commercial antibiotics.

The compounds were synthesized according to our reported methods with minor modifications (Scheme 1). The compounds for the current study were synthesized using 1,3,5-tris(bromomethyl)benzene. The mono-modification of 1,3,5-tris(bromomethyl)benzene with azide and pyridine resulted compounds **2** and **3**, which were further modified with aliphatic thiols to provide compounds **4** and **5**, respectively. The azide-alkyne click reaction of compound **4** with prop-2-yne-1-sulfonic acid produced the compound **6**. Finally, the treatment of compounds **5** and **6** with methyl iodide provided the desired product **7** and **8** with satisfactory yield. These compounds were characterized by nuclear magnetic resonance (NMR), high-resolution mass spectrometry (HRMS).

The sulfonium-based compounds showed antibacterial activity against both gram-positive and gram-negative bacteria. The minimum inhibitory concentrations (MIC) of the compounds were calculated against gram-negative bacteria such as *Escherichia coli* MTCC 1687 (*E. coli*) and *Pseudomonas aeruginosa* MTCC 2488 (*P. aeruginosa*) and gram-positive bacteria such as *Staphylococcus aureus* MTCC 96 (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA) by micro broth dilution method. The MIC values were reckoned as the minimum concentration of the compounds, which caused no visible growth, or the optical density (OD) of the compound treated bacterial culture was close to that of control (without any bacteria). The compounds **7a** and **8** showed very good antibacterial activity against all the four tested bacterial strains (Table 1). The MIC values of the pyridinium containing compound **7a** were within 6.3-12 μM. It was emboldening to observe that the drug resistant strain of *S. aureus* showed similar MIC value to that of its drug sensitive strain. Whereas, the sulfonic acid containing compound **8** had little higher MIC values of 12-24 μM.

Further antibacterial analysis was done to investigate the impetus of different structural moieties in the compound, which provides it antimicrobial property. Compound **4** with azide but no sulfonium moieties showed no antibacterial activity even at 100 μM. Compound **5a** with only pyridinium but no sulfonium moieties showed no antibacterial activity even at 100 μM, suggesting the role of sulfonium moieties in the antibacterial activities of compound **7a**. Compound **7b** with same head group, but with short alkyl chain (ethyl group) length to that compound **7a**, showed no antibacterial activity even at 100 μM, suggesting that the long hydrocarbon chain is as important as other moiety in the compound. Compound **5b** with short alkyl chain length also showed no antibacterial activity. Probably, the sulfonium and pyridinium or sulfonic acid moieties could be involved in electrostatic or hydrogen bond interactions with the phosporyl and carboxyl groups of the bacterial membrane lipids and elicit the incretion of the long hydrocarbon chain lengths of compounds **7a** and **8** to the bacterial membrane. However, the higher antibacterial activity of compound **7a** over **8** could be due to much stronger electrostatic interaction of pyridinium moiety with anionic lipids of the bacterial membrane than that with the sulfonic acid moiety.

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