Development of Novel Antimicrobial Agents to Prevent Medical Device Related Infections

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Introduction

Medical device associated infections remain a serious health issue. *Staphylococcus aureus* is one of the most frequently isolated bacteria from infected medical devices. New non-antibiotic antimicrobial agents are needed due to drug resistance of many clinical strains and biofilm formation. Transition metal iron (Fe) is essential for growth of all pathogens, so disruption of bacterial Fe metabolism is becoming a novel anti-infective approach [1]. Due to their chemical similarity to Fe, gallium (Ga) and zinc (Zn) can substitute for Fe in many biological systems, but do not undergo redox cycling, and thus inhibit Fe-dependent processes. It has been demonstrated that free Ga^{3+} is very effective in preventing growth and biofilm formation of Gram-negative *Pseudomonas aeruginosa* [2]. However, free Ga^{3+} does not work directly on Gram-positive *S. aureus*. Here two antibacterial agents that render Ga and Zn effective in disrupting metabolism of *S. aureus* are reported.

Materials and Methods

$Ga(NO_3)_3$, protoporphyrin IX (PPIX) and PPIX-Zn were purchased from Sigma-Aldrich. Tryptic soy broth (TSB) was purchased from Fisher Scientific. 1% TSB (0.3g/L) was used in all experiment unless otherwise defined. PPIX-Ga was prepared by refluxing PPIX and $Ga(NO_3)_3$ in dimethylformamide for 24 hours under Argon atmosphere [3].

Two *S. aureuss* strains, SA12600 and SA MN8, were tested. Organisms were cultured in 10% TSB at 37°C overnight, isolated and then rinsed with 1% TSB prior to use. In the planktonic study, isolated bacteria were inoculated into sterile plastic tubes at a concentration of 1.5×10^5 cells/ml, and incubated with or without antimicrobial agents at 37°C for 24 hours. The colony forming units were counted by using TSB agar plates.

Cytotoxicity of these antimicrobial agents was tested on NIH/3T3 fibroblast cells. When the cells were cultured to subconfluecy, the agents were added. Their effects on cell growth and morphology were observed under microscope after 24-hour incubation at 37°C and 5% CO_2.

Results and Discussion

Unlike prior reports for *P. aeruginosa* [1], free Ga^{3+} was unable to effectively inhibit growth of *S. auerus*. As shown in Figure 1a, Ga^{3+} had a slightly inhibitory effect on both SA12600 and SA MN8 at a concentration of 50 μM. So, while levels in excess of 50 μM Ga^{3+} are needed to completely prevent SA growth, only 10 μM Ga^{3+} can completely inhibit growth of *P. aeruginosa* PAO1 [1]. When tested alone, metal free porphyrin did not affect *S. auerus* growth either (Figure 1b). However, when complexed with Ga or Zn, PPIX completely prevents SA growth and even kills planktonic SA12600 and SA MN8 at very low concentrations (Figures 1c & 1d). Given the starting cell concentration of 1.5×10^5 cells/ml, any colony forming units (CFU) lower than this number means complete inhibition of bacterial growth. For both SA12600 and SA MN8, 3.2 nM PPIX-Ga or PPIX-Zn was sufficient to prevent their growth, and PPIX-Ga completely killed SA12600 at 3.2 nM (2 μg/mL) and SA MN8 at 16 nM (10 μg/mL).

Cytotoxcicity results indicated that free Ga^{3+}, PPIX alone and PPIX-Ga did not affect growth and morphology of 3T3 fibroblast cells at all the concentrations used in bacterial growth study (data not shown). However, PPIX-Zn showed slightly toxic effects on the 3T3 cells at concentrations above 16 nM. Whether the toxicity of PPIX-Zn was due to the compound nature or due to impurities needs further investigation.

PPIX is a prosthetic group of hemoglobin or other hemoproteins. It is suggested that non-iron metalloporphyrins disrupted bacterial metabolism by using their haem uptake systems as portals of entry [4].

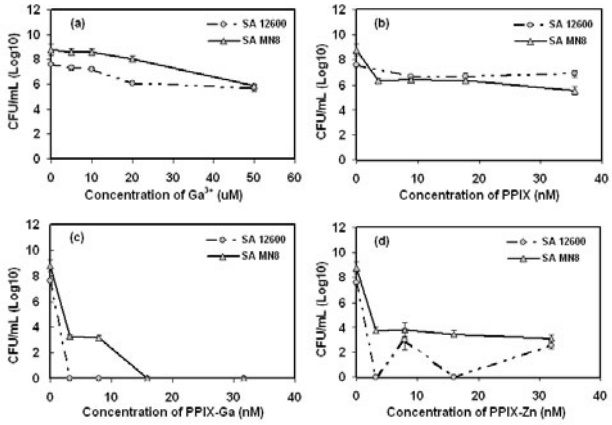

Figure1. The effects of Ga^{3+} (a), PPIX (b), PPIX-Ga (c) and PPIX-Zn (c) on growth of planktonic *S aureus*.

Conclusions

Protoporphyrin IX complexes with Ga and Zn are very effective in preventing growth of planktonic *S aureus*. PPIX-Ga even killed *S aureus* at a low concentration and did not exhibit cytotoxicity. PPIX-Ga was a promising non-anticbiotic therapeutic that could be released from a biomaterial thus preventing *S. aureus* associated device infections.

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References

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