Introduction

Otitis Media (OM) is a general term that describes infections and inflammatory responses of the middle ear. Due to its diverse causes and clinical manifestations, OM represents a serious challenge. The advent of tympanostomy tubes (TTs) significantly decreased the middle ear. One of the potential novel antimicrobial agents is Next science wound gel technology, a novel agent that inhibits biofilm development by gram-positive and gram-negative bacteria. Recent studies have demonstrated that antimicrobial agents are effective in inhibiting biofilm development by different wound pathogens. In the present study, we report experiments that demonstrate the efficacy of NS in killing Pa and Sa planktonic bacteria as well as inhibiting the development of Pa and Sa biofilms on TTs.

Materials and Methods

1. Bacterial strains: The Pseudomonas aeruginosa strain PA01, which carries the red fluorescence plasmid pMP105 and the S. aureus strain ATCC29213 which carries the green fluorescent plasmid pMP705, were used. Bacterial strains were grown overnight at 37°C in LB broth containing the appropriate antibiotic (for PA01: pMP105 and erythromycin for ATCC29213).

2. Minimum inhibitory concentrations (MICs): The MIC analysis, which was done as previously described in Figure 2, was conducted to determine the amount of NS agent required to inhibit the planktonic growth of the tested strains (PaMIC = 325 μg/mL, SaMIC = 203125 μg/mL).

3. The static biofilm model: Biofilms were developed on TTs using a previously described model. Details of the model are shown in Figure 1.

4. Biofilm analysis: The biofilms were quantified by determining the number of microorganisms (CFU/tube) within each biofilm as described in Materials and Methods. Values represent the average of three independent experiments ± SEM. Values are considered statistically significant when p < 0.05.

Results

Figure 1. Diagram depicting the process of biofilm development on TT and the quantitation (CFU/tube) of each biofilm. The details of biofilm development and biofilm analysis are described in Materials and Methods.

Figure 2 (A & B). MIC. The Next Science (NS) minimum inhibitory concentration (MIC) for the planktonic culture of P. aeruginosa is considerably greater than that of S. aureus (325 μg/mL) and Sa203125 μg/mL (B). Overnight cultures of either Pa or Sa were diluted in 1X PBS to an optical density (OD600) of 0.22 and 0.42, respectively and subcultured in the diluted TSB as described in Materials and Methods. Various concentrations of NS were then added to several tubes of each bacterial culture, and the tubes were incubated under shaking conditions in a 37°C waterbath. The highest dilution of NS that completely inhibited the growth of the tested bacteria is considered the MIC. Each experiment was repeated three times. Values represent the average of at least three independent experiments ± SEM.

Figure 3 (A & B). At 325 μg/mL, Next Science (NS) inhibited the development of Pa biofilms on the inner and outer surfaces of the TTs. Biofilm development on TTs was conducted as described in the Materials and Methods section, in the presence or absence of NS. After 24 hours of incubation at 37°C, the tubes were rinsed to remove loosely attached bacteria and examined. (A) The biofilms were quantified by determining the number of microorganisms (CFU/tube) within each biofilm (as described in Materials and Methods). Values represent the average of three independent experiments ± SEM. (B) Biofilm development on the inner and outer surface of the TT was visualized using confocal laser scanning microscopy (CLSM). Plasmid pMP705, which codes for the red fluorescent protein, was transformed into the strain PA01.

Figure 4 (A & B). At 3203125 μg/mL, Next Science (NS) inhibited the development of Sa biofilms on the inner and outer surfaces of the TTs. Biofilm development on TTs was conducted as described in the Materials and Methods section, in the presence or absence of NS. After 24 hours of incubation at 37°C, the tubes were rinsed to remove loosely attached bacteria and examined. (A) The biofilms were quantified by determining the number of microorganisms (CFU/tube) within each biofilm (as described in Materials and Methods). Values represent the average of three independent experiments ± SEM. (B) Biofilm development on the inner and outer surface of the TT was visualized using confocal laser scanning microscopy (CLSM). Plasmid pMC1, which codes for the green fluorescent protein, was transformed into the strain SA24013.

Conclusions

Our results suggest that: (1) through its ability to inhibit the planktonic growth as well as the biofilm development of two major otopathogens (Pa and Sa), NS is a potential antimicrobial agent to treat middle ear infections; (2) the dose of NS required to inhibit the planktonic growth and biofilm development by each pathogen varies. Currently, we are investigating the efficacy of NS in preventing and treating other otopathogens.

References

6. Vos-Dengel TM, van der Heijden GJ, van der Heijden GJ, van der Heijden GJ, Venekamp RP, et al. A trial of treatment for acute otorrhea in children with tympanostomy tubes for 8 hours post-inoculation, one set of the biofilms was incubated further for an additional 24 hours (Control group). NS was added to the partial biofilms (5.25 μg/mL) for Pa and 3.26 μg/mL for Sa and the incubation was continued for an additional 24 hours (Experimental group). Afterwards, the biofilms (Control and Experimental groups) were rinsed, and the number of microorganisms per TT in each biofilm (CFU/tube) was determined for Pa and Sa. Values represent the average of three independent experiments ± SEM.