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Activity of OligoG Alginate Against Gram-Positive Bacteria, Alone and in Combination with Anti-Gram Positive Antibiotics

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Background. OligoG is an oligosaccharide derived from alginate polysaccharide. Initial studies indicate that OligoG disrupts biofilm formation and thus could be used as an effective antimicrobial; therefore we examined the activity of OligoG against Gram-positive bacteria alone, and in combination with standard Gram-positive antibiotics and the ability of OligoG to disrupt MRSA biofilms.

Methods. Twelve isolates; six MRSA isolates with various multi-drug resistant (MDR) profiles, an MDR *Streptococcus oralis* and *Enterococcus faecium*; non-MDR strains of *Staphylococcus aureus* (NCTC 6571 and ATCC 6538); *S. oralis* and *E. faecium* were tested against 12 antibiotics (4 macrolides, 2 aminoglycosides, 3 β-lactams, tetracycline, ciprofloxacin and colistin) with increasing concentrations of OligoG. OligoG on the bacteria was studied using scanning electron microscopy (SEM) and live/dead staining of more mature 6h biofilms.

Results. The addition of OligoG to the aminoglycosides increased the MICs 2-16 fold for all isolates indicating non-specific binding between the two molecules. However, with the non-MRSA Gram-positive bacteria, OligoG showed a marked decrease in MICs with macrolides (4-256-fold), β-lactams (2-8-fold) and ciprofloxacin (2-16-fold). The MIC values of the MICs were very high and 10% OligoG had little effect on lowering these. However, MRSA strain, U50, showed potentiation with most antibiotics at 6% and 10% OligoG. Tetracycline and colistin showed no change in the MIC values. SEM showed that at 6 and 10% there was significantly less (70% deduction) MRSA adhered to the 6h biofilms.

Conclusion. OligoG shows synergistic activity with macrolides, β-lactams and ciprofloxacin against most Gram-positive bacteria which is supported by SEM biofilm analysis showing decreased biomass. OligoG could play a supportive role in combination therapy for MRSA biofilms.

INTRODUCTION

OligoG is an oligosaccharide derived from alginate polysaccharide. Initial studies indicate that OligoG disrupts biofilm formation and thus could be used as an effective antimicrobial; therefore we examined the activity of OligoG against Gram-positive bacteria alone, and in combination with standard Gram-positive antibiotics and the ability of OligoG to disrupt MRSA biofilms.

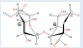

Figure 1. Structure of the repeating guluronate units of the OligoG molecule

AIMS & OBJECTIVES

This study investigated a novel treatment strategy to determine if the susceptibility of gram-positive species to antibiotic/antimicrobial therapy could be modulated by pre-treatment with defined alginate oligomers (OligoG CF-5/20).

MATERIALS & METHODS

Twelve isolates; six MRSA isolates with various multi-drug resistant (MDR) profiles, an MDR *Streptococcus oralis* and *Enterococcus faecium*; non-MDR strains of *Staphylococcus aureus* (NCTC 6571 and ATCC 6538); *S. oralis* and *E. faecium* were tested against 12 antibiotics (4 macrolides, 2 aminoglycosides, 3 β-lactams, tetracycline, ciprofloxacin and colistin) with increasing concentrations of OligoG. The effect of OligoG on the bacteria was studied using scanning electron microscopy (SEM) and live/dead staining.

RESULTS

MIC DETERMINATIONS

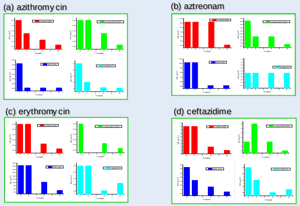

Figure 2. MIC determinations for Gram-positive isolates when cultured with 0, 2, 6 or 10% OligoG against six antibiotics **(n=8)**

Antibiotic	% OligoG	*S. aureus* 9518	*S. aureus* NCTC 6571	*S. aureus* MRSA 1040	*Strep. oralis* 5610
Azithromycin	0	0.5	0.25	512	8
	2%	0.25	0.25	256	2
	6%	0.125	0.125	256	<0.25
	10%	0.03125	<0.03125	256	<0.25
Erythromycin	0	0.25	0.25	>1024	2
	2%	0.25	>16	>1024	16
	6%	0.0625	0.0625	1024	0.03125
	10%	<0.03125	<0.03125	512	0.0625
Roxithromycin	0	0.5	0.5	>1024	8
	2%	0.25	0.25	1024	8
	6%	0.5	0.25	512	0.03125
	10%	0.0625	0.0625	256	16
Dirithromycin	0	0.5	0.5	1024	4
	2%	0.5	0.5	512	4
	6%	0.25	0.125	512	<0.03125
	10%	0.0625	0.03125	256	0.03125
Aztreonam	0	512	512	1024	<0.5
	2%	512	256	1024	<0.5
	6%	512	256	512	<0.5
	10%	512	128	512	<0.5
Ceftazidime	0	8	4	>16	8
	2%	8	8	16	4
	6%	2	4	8	0.03125
	10%	1	2	2	1

Table 1. Graphs showing MIC determinations for four Gram-positive organisms with OligoG at 0, 2, 6 and 10%. (Results for 4 isolates & 4 antibiotics are shown)

The antibiotics azithromycin, erythromycin, dirithromycin, roxithromycin, aztreonam and ceftazidime all showed potentiation of antibiotic activity in the presence of increasing concentrations of OligoG against Gram-positive isolates including MRSA

ADHERENCE ON TITANIUM DISCS

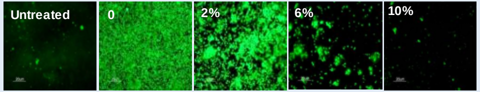

Figure 3. Adherence of *Oxford Staphylococcus* on titanium discs pre-coated with 0, 2, 6 or 10% OligoG. (The untreated control was uninoculated)

Bacterial adherence of *S. aureus* on titanium discs decreased when coated with an increasing concentration of OligoG

LIVE-DEAD STAINING

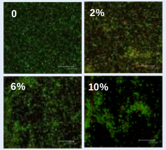

Figure 4. Live-dead staining of *S. aureus* biofilms (24 h) generated in the presence of 0, 2%, 6% or 10% OligoG

An increase in dead cells and in clumping of cells was seen with increasing OligoG concentration

CONCLUSIONS

- Little or no synergistic activity was found with the following antibiotics; tobramycin, amikacin, imipenem, ciprofloxacin, colistin or oxytetracycline (results not shown)
- However, OligoG showed synergistic activity with macrolides and β-lactams against most Gram-positive bacteria tested including MRSA
- This is supported by adherence and live-dead biofilm analysis showing decreased biomass with increasing concentration of OligoG
- OligoG may play a supportive role in combination therapy for MRSA biofilms

REFERENCES

1. Jorgensen et al., (1999) *Manual of Clinical Microbiology*, 7th ed. Washington, D.C: ASM, pp.1526-1543

ACKNOWLEDGEMENTS

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