

Hill, Katja E¹; Khan, Saira¹; Onsøyen, Edvar²; Myrvold, Rolf²; Dessen, Arne²; Walsh Timothy R³; Howe, Robin A⁴; Thomas, David W¹

¹Wound Biology Group, Cardiff Institute of Tissue Engineering and Repair, Cardiff University School of Dentistry, Cardiff, UK CF14 4XY, ²AlgiPharma AS, Industriveien 33, N-1337 Sandvika, Norway;

³Department of Medical Microbiology, School of Medicine, Cardiff CF14 4XN; ⁴Medical Microbiology (Velindre NHS Trust), University Hospital of Wales, Cardiff UK.

INTRODUCTION

Pseudomonas aeruginosa (PA) is a common cause of morbidity in cystic fibrosis patients. An important feature of the disease is the increasing incidence of multiple antimicrobial resistance (MDR) to the antibiotics most commonly employed in treatment. Resistance may be potentiated by the bacterial biofilm, containing extracellular polysaccharide (1). Alginates are polysaccharides produced by PA. Alginates are linear polymers of (1-4) linked α -D-mannuronic acid (M) &/or its C-5 epimer α -L-guluronic acid (G).

Previous studies have demonstrated that mannuronic acid polymers were able to modulate immunological responses (2) and to reduce the viscosity of CF sputum, facilitating its disruption (Fig 1; (3)). In this study we sought to investigate the potential of alginate oligomers to modulate biofilms, and whether this may be reflected in an ability to modify the resistance of PA to antibiotic therapies.

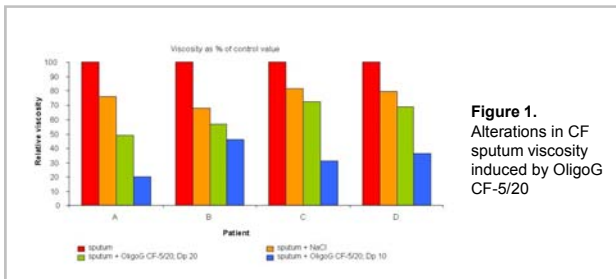


Figure 1. Alterations in CF sputum viscosity induced by OligoG CF-5/20

AIMS & OBJECTIVES

These studies investigated the effect of OligoG CF-5/20 treatment to modulate the interaction of *Pseudomonas* strains with antibiotics employed in the treatment of CF in both conventional Minimum Inhibitory Concentration (MIC) assays against planktonic bacteria and in a Minimum Biofilm Eradication Concentration (MBEC) assays against bacterial biofilm assembly.

MATERIALS & METHODS

These studies utilised *Pseudomonas* reference strains from culture collections and clinical isolates. OligoG CF-5/20 is an oligomer derived from alginate by AlgiPharma AS and is currently under clinical investigation for the treatment of CF.

Antimicrobial activity was assessed in standard MIC (4) and MBEC (5) assay systems as previously described. The effect of the OligoG CF-5/20 on bacterial biofilm populations was studied using scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) using cell membrane dye FM[®] 1-43 SE (Invitrogen) and live/dead staining (LIVE/DEAD[®] BacLight[™] Bacterial Viability Kit, Invitrogen).

RESULTS

Table 1. Treatment of planktonic PA cultures with OligoG CF-5/20 lowers the MIC values of antibiotics used in combination with varying concentrations of OligoG CF-5/20 (0-10%). The magnitude of the effect was pronounced for the PA strain R22 & the potentiation of the antibiotic Azithromycin with all strains studied.

Antibiotic	% OligoG CF-5/20	PA01	Mucoid PA ATCC 39324	Mucoid PA CFA 24-1	PA R22	PA 301
Meropenem	0	2	<1	<1	32	64
	2%	2	<1	<1	32	64
	6%	<1	<1	<1	16	128
	10%	<1	<1	<1	4	128
Ceftazidime	0	<1	<1	<1	128	32
	2%	<1	<1	<1	64	16
	6%	<1	<1	<1	32	8
	10%	<1	<1	<1	8	4
Aztreonam	0	8	2	<1	32	64
	2%	16	2	<1	16	16
	6%	4	<1	<1	<4	8
	10%	2	<1	<1	<4	8
Azithromycin	0	128	128	256	64	64
	2%	64	64	128	64	64
	6%	16	16	64	64	32
	10%	4	4	8	32	16
Ciprofloxacin	0	0.125	0.125	1	16	16
	2%	0.0625	0.0625	0.125	16	16
	6%	0.0625	0.03125	0.125	8	8
	10%	0.03125	0.03125	0.125	4	8

PA01, PA type strain; PA ATCC 39325, mucoid type strain, PA CFA 24-1, clinical mucoid strain; PA R22, PA MDR strain from Hunan, China; PA 301, MDR strain from Warsaw, Poland

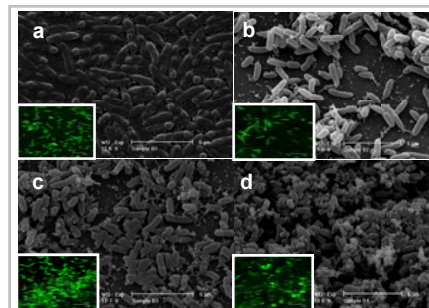


Figure 2. SEM pictures of the 6hr-treated PA01 biofilms incubated with varying concentrations of OligoG CF-5/20; (a) 0, (b) 2%, (c) 6% or (d) 10%. Treatment with increasing OligoG CF-5/20 concentrations was associated with disruption of the biofilms. CLSM of 6hr-treated PA01 biofilms (insets) demonstrated alteration of bacterial morphology.

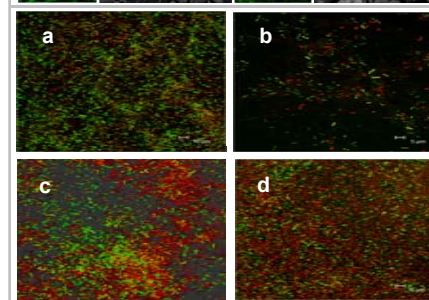


Figure 3. LIVE-DEAD staining of 6hr PA01 biofilms treated with (a) 0, (b) 2%, (c) 6% or (d) 10% OligoG CF-5/20. Increased numbers of dead cells (RED) were evident with increasing OligoG CF-5/20 concentration.

Table 2. Summary of MBEC values at 6 h after re-growth following overnight exposure to antibiotic of PA01 biofilms pre-treated overnight with 0 or 6% OligoG CF-5/20. Values in brackets represent fold reduction in MBEC value compared to the 0% OligoG CF-5/20 control.

Antibiotic	MBEC values at 6 h ($\mu\text{g ml}^{-1}$)	
	0% OligoG CF-5/20	6% OligoG CF-5/20
Amikacin	512	128 (2)
Tobramycin	32	8 (2)
Oxytetracycline	512	64 (3)
Ceftazidime	4096	1024 (2)
Ciprofloxacin	128	32 (2)
Amikacin + Tobramycin	4	2 (1)
Amikacin + Oxytetracycline	128	64 (2)

CONCLUSIONS

- OligoG CF-5/20 can potentiate the activity of antibiotics against *Pseudomonas* sp. inhabiting the CF lung.
- This potentiation is evident in both planktonic and biofilm conditions.
- Treatment with OligoG CF-5/20 may represent a useful adjunct to conventional antimicrobial therapy in CF and other patients with Gram-negative infections.

REFERENCES

- Aaron et al., *J Clin Microbiol* 2002, 40:4172-9
- Flo et al., *J Biol Biochem* 2002, 277:35489-95
- Draget et al., *Use of Oligouronates for Treating Mucus Hyperviscosity*, US Patent Application 20090010914, 2009
- Jorgensen et al., *Manual of Clinical Microbiology*, 7th ed. Washington, D.C: ASM, 1999, 1526-43
- Moskowitz et al. *J Clin Microbiol* 2004, 42:1915-22

ACKNOWLEDGEMENTS

This work was sponsored by AlgiPharma AS.