

Investigation into the potential of sub-lethal PACT to reduce antibiotic susceptibility

C. M. Cassidy,

R. F. Donnelly, P. A. McCarron and M. M. Tunney

Presentation Outline

- Introduction to topic (background)
- Aim and objectives
- Materials and Methods
- Results
- Discussion
- Further work planned

Introduction: Photodynamic Antimicrobial Chemotherapy (PACT)

- Use of non-toxic, highly conjugated photosensitizers (PS) + light
- Excitation of PS molecules on irradiation leads to the production of **Reactive Oxygen Species** (ROS)
- Toxic to microorganisms and cause cell death by a variety of non-specific methods

Aim

- To determine if exposure to sub-lethal PACT can reduce susceptibility of bacteria to
 - Antibiotics
 - PACTto which they were previously susceptible

Objectives

- To determine lethal and sub-lethal PACT conditions for representative Gram-positive and Gram-negative bacteria
- To expose bacteria to sub-lethal PACT conditions
- To determine if exposed bacteria differ from non-exposed bacteria with respect to antibiotic and PACT susceptibility

Materials

- Photosensitizers (PS) used
 - Methylene blue (MB) (Sigma aldrich, UK)
 - meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) (Frontier Scientific, UK)
- Strains used
 - *Pseudomonas aeruginosa* (PAO1) = Gram negative
 - *Staphylococcus aureus* (AH7) = Gram positive
- E-tests[®] (AB Biodisk, Sweden)
- Light source
 - Paterson lamp (Photo Therapeutics, Cheshire, UK)

Sub-lethal PACT

Combination of PS and light that does not cause complete kill

- Light dose kept constant at 100 J cm^{-2}
- PS concentration associated with sub-lethal and lethal killing determined

Methods

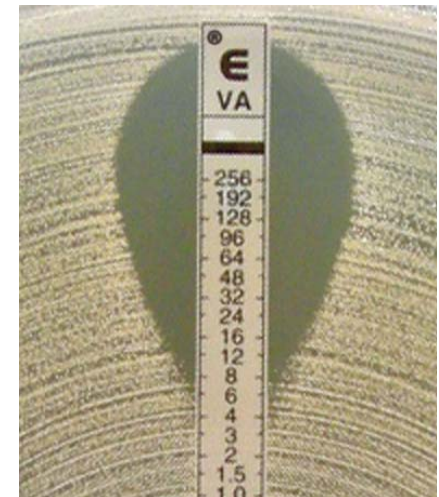
- Planktonic culture of bacteria
- Samples:
 - S-L- control = Mueller Hinton broth (MHB) only
 - S+L- control = MHB with PS adjusted to sub-lethal concentration
 - S+L+ test = sub-lethal PS + MHB, irradiated at 24 hour intervals

Methods

- At $t = 0, 24$ and 48 hours:
 - 100 μl samples exposed to sub-lethal PACT or equivalent control conditions
- At 72 hours, susceptibility to a range of antibiotics and previously lethal PACT tested

Susceptibility testing

- E-tests[®] used to determine the Minimum Inhibitory Concentration (MIC) of selected antibiotic
- MIC = Minimum concentration of antibiotic required to inhibit visible growth of microorganism



Results- lethal and sub-lethal PACT

- Lethal concentrations of PS for
 - PAO1 & AH7
 - TMP & MB= 250 $\mu\text{g ml}^{-1}$
- Sub-lethal concentrations of PS for
 - PAO1 & AH7
 - TMP & MB= 25 $\mu\text{g ml}^{-1}$

Results: *P.aeruginosa* (PAO1)

- No change in MIC
 - Piperacillin/tazobactam
 - Tobramycin
- Increase in MIC
 - Ceftazidime (TMP-PACT)
 - Meropenem (MB- and TMP-PACT)

Results: *S.aureus* (AH7)

- No change in MIC
 - Fusidic acid
 - Linezolid
- Increase in MIC
 - Vancomycin (TMP-PACT)
 - Mupirocin (MB- and TMP-PACT)

Results: Previously lethal PACT

- Comparison @ 72 hours
 - S-L- and S+L+ samples
 - Exposed both to previously lethal PACT
- No decrease in efficacy of lethal PACT
 - Trend = % kill S-L- < % kill S+L+

% kill		Run 1		Run 2	
		S-L-	S+L+	S-L-	S+L+
PAO1	MB	38.46	88.46	73.00	87.00
	TMP	25.64	91.15	99.99	99.99
AH7	MB	45.80	97.02	99.99	99.76
	TMP	50.00	96.67	100.00	100.00

Discussion

- Exposure to sub-lethal PACT provides oxidative stress in the absence of cell death
- In response to oxidative stress, bacteria can up-regulate oxidative stress genes [1] and antibiotic resistance genes
- The up-regulation of these genes and potential transfer of genetic material may result in a resistant bacterial population

[1] Cosgrove *et al.* J Bacteriol 189:3 (2007) 1025 -35

Discussion

- “Reading of E-tests[®] can be both subjective and variable” [2]
- Changes in MIC were not significant
 - Did not cross breakpoints between resistant and susceptible for ANY organism / antibiotic tested
 - Crossed greater than one doubling dilution on only ONE occasion
 - Ceftazidime after exposure to sub-lethal TMP-PACT
 - MIC ↑ from 0.75 → 3 µg ml⁻¹

[2] Ge, Bodeis, Walker *et al.* J Antimicrob Chemoth 50 (2002) 487-494.

Conclusion

- Exposure to sub-lethal PACT conditions does not infer resistance to:
 - Previously lethal PACT
 - Antibiotics tested for both strains

Further work

- Complete susceptibility testing on a range of clinical isolates
 - *Pseudomonas aeruginosa*
 - *Staphylococcus aureus*
 - MRSA
 - MSSA

Acknowledgements

- Support
 - Stef Mc Grath
 - Dr. Deirdre Gilpin
 - Co-workers in QUB
- Funding provided by Department for Employment and Learning, Northern Ireland (DEL)

Questions

