Photodynamic antimicrobial chemotherapy coupled with the use of the photosensitizers methylene blue and temoporfin as a potential novel treatment for Staphylococcus aureus in burn infections

Alistair Hampden-Martin, Jo Fothergill, Mohamed El Mohtadi, Lucy Chambers, Anthony J. Slate, Kathryn Whitehead, Kayvan Shokrollahi

Poster by Laura Cappuyns

Mersey Burns Centre, Whiston Hospital, Warrington Road, Prescot, Merseyside, L35 5DR
Manchester Metropolitan University

Introduction

Widespread antibiotic misuse, coupled with an increasingly mobile global population, has facilitated an alarming increase in the rates of emerging antimicrobial-resistant (AMR) bacteria. In Europe alone, multidrug-resistant (MDR) bacteria are estimated to be responsible for ~25,000 deaths per year (1). Commonly isolated AMR bacteria from patients include:

- methicillin-resistant Staphylococcus aureus (MRSA)
- vancomycin-resistant Enterococcus spp. (VRE)
- carbapenem-resistant Enterobacteriaceae spp.
- MDR Pseudomonas spp.

Photodynamic antimicrobial chemotherapy (PACT) is a novel alternative antimicrobial therapy that elicits a broad mechanism of action and therefore has a low probability of generating resistance. The antimicrobial effect of PACT relies on 3 components: the presence of oxygen (O2), a photosensitizer and a wavelength of light that coincides with the peak absorption of the photosensitizers (2).

Burns patients are at high risk of nosocomial infection due to compromised innate host defences. Bacterial colonization of burns can result in invasive infection, septicaemia, multi-organ failure and death. Novel therapies to treat burn infections are urgently needed; particularly therapies that will not facilitate the development of antimicrobial resistance. One potential avenue to be explored is PACT.

AIM: This study aimed to assess the antimicrobial efficacy of methylene blue (MB) - and temoporfin-mediated PACT against both Gram+ve and Gram-ve bacterial species (namely *S. aureus* & *P. aeruginosa*) that are commonly isolated from burn infections.

Method

1) **Bacterial culture** - S. aureus & P. aeruginosa bacteria were cultured aerobically

2) **Photosensitizers and light source**

- **Photosensitizers:** Methylene blue (MB) and Temoporfin were used. Both photosensitizers were stored in a dark environment to minimize light exposure prior to experimentation.
- **Light source:** portable light-emitting diode (LED) PDT light source that had a red wavelength (λ) (640 nm)

3) **PACT assays** - Experiments were conducted in clear, flat-bottom, 96-well microtitration plates.

- *S. aureus* and *P. aeruginosa* were exposed to 4 different parameters in the presence of both MB and temoporfin, and red light. A maximal light exposure time of 20 min was used, due to the assumption that patients would tolerate longer treatment times poorly.
- All PACT experiments were conducted in triplicate alongside a LB broth (negative control).

Statistical analysis

2-way analysis of variance (ANOVA) coupled with Tukey's multiple comparison tests for post hoc analysis using GraphPad Prism to determine significant differences at a confidence level of 95 % ($P<0.05$).

- Error bars represent the standard error of the mean.
- Asterisks denote significance, *P≤0.05, **P≤0.01, ***P≤0.001 and ****P≤0.0001

Results

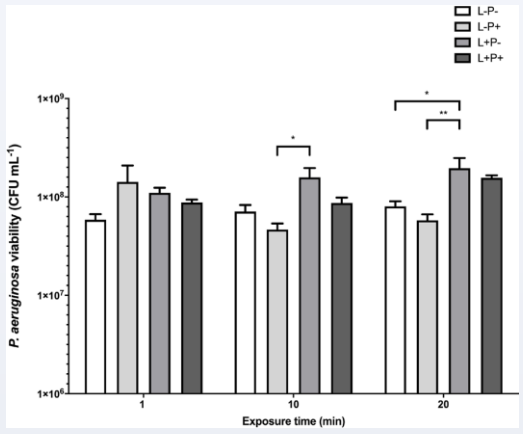

Fig. 1. Effect of MB (1 mg ml−1) on P. aeruginosa (B9T2436) after 1, 10 and 20 min of red light exposure (λ=640 nm; n=3). Group L+P+, incubated with MB for 20 min, and then irradiated with red light. Group L+P−, no incubation with MB but exposed to red light. Group L−P−, no incubation with MB or exposure to red light. Group L−P+, incubated with MB, but not exposed to red light. Bars represent median value +range of three biological replicates. Two-way ANOVA tests were performed between experimental groups at different time points. Asterisks denote significance (*P≤0.05, **P≤0.01).

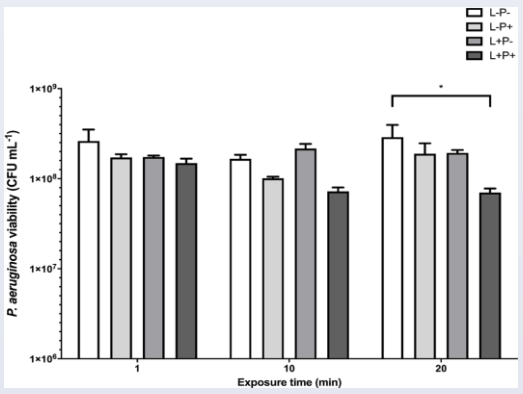

Fig. 2. Effect of Temoporfin (50 μM) on *P. aeruginosa* (B9T2436) after 1, 10 and 20 min of red light exposure (λ=640 nm; *n*=3). Group L+P+, incubated with Temoporfin for 20 min, and then exposed to red light. Group L+P−, not incubated with temoporfin but exposed to red light. Group L−P−, not incubated with temoporfin or exposed to red light. Group L−P+), incubated with temoporfin but not exposed to red light.
Bars represent the mean of three biological replicates whilst error bars denote standard error of mean (sem). Two-way ANOVA tests were performed between experimental groups at different time points.Asterisks denote significance (*P≤0.05).

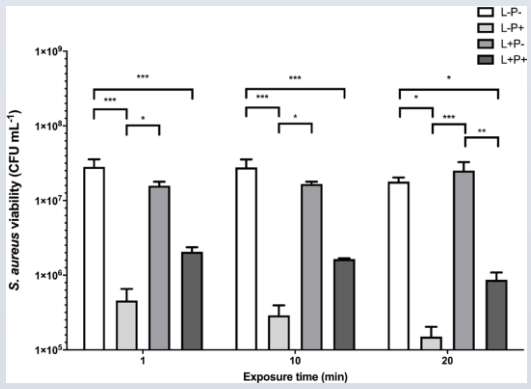

Fig. 3. Effect of MB (1 mg ml−1) on S. aureus c.f.u. ml−1 after 1, 10 and 20 min of red light exposure (λ=640 nm; n=3). Group L+P+, incubated with MB for 20 min, and then exposed to red light. Group L+P−, no incubation with MB but exposed to red light. Group L−P−, no exposure to MB and no exposure to red light. Group L−P+, incubation with MB but no exposure to red light. Bars represent the mean of three biological replicates whilst error bars denote standard error of mean (sem). Two-way ANOVA tests were performed between experimental groups at different time points. Asterisks denote significance (*P≤0.05, **P≤0.01 and ***P≤0.001).

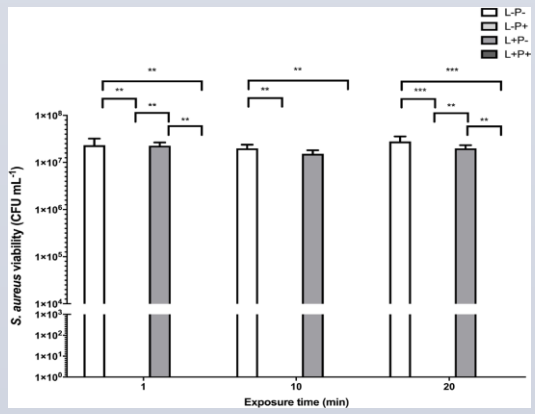

Fig. 4. Effect of Temoporfin (12.5 μM) on *S. aureus* c.f.u. ml−1 after 1, 10 and 20 min of red light exposure (λ=640 nm; *n*=3). Group L+P+, incubated with temoporfin for 20 min, and then exposed to red light. Group L+P−, no incubation with temoporfin but exposed to red light. Group L−P−, no incubation with temoporfin or exposure to red light. Group L−P+, incubated with temoporfin but not exposed to red light. Bars represent the mean of three biological replicates whilst error bars denote standard error of mean (sem). Two-way ANOVA tests were performed between experimental groups at different time points. Asterisks denote significance (*P≤0.05, **P≤0.01 and ***P≤0.001).

Discussion

This study explored the efficacy of light-activated photosensitizers against bacterial species commonly found in burn wound infections (see results summary in figure 5). The results from this *in vitro* study demonstrated that *S. aureus* was more susceptible to killing by the photosensitizers in the absence of light than *P. aeruginosa*.

Temoporfin demonstrated greater antimicrobial efficacy than MB against *S. aureus* isolate and *P. aeruginosa* isolate tested in vitro. Therefore antimicrobial activity of Temoporfin as a photosensitizer could be more suited to Gram +ve bacterial infections.

The greater sensitivity of Gram +ve bacteria to photosensitizers has been reported by other in vitro studies. The discrepancy in sensitivity is believed to be due to differences in cell wall structure, with Gram –ve bacteria having an additional negatively charged outer membrane that impedes the diffusion of non-cationic photosensitizers (3).

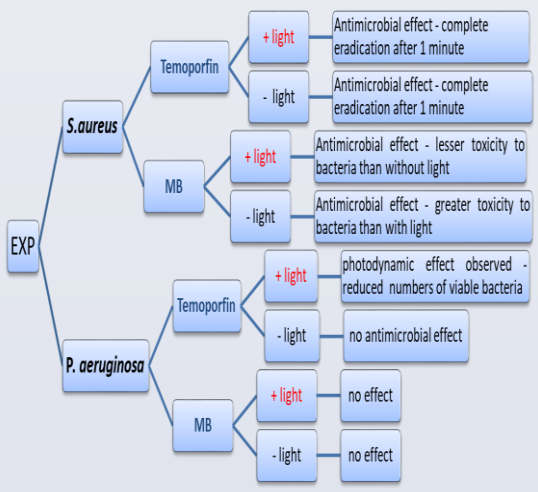

Fig. 5 Summary of effect of Temoporfin and MB – mediated PDT on *S. aureus* & *P. aeruginosa*

Conclusions

Photodynamic therapy against bacteria is demonstrably effective

- PACT eradicated *S. Aureus* completely in this study.
- PACT was effective at reducing the quantity of *P. Aeruginosa* but not as effectively as against *S. Aureus*.

The role of PACT needs further investigation and has great potential in the future management of burn patients.

Recommendations

In light of this study, further research into the validity of PACT, coupled with the photosensitizers (such as Temoporfin), should be conducted in order to potentially develop alternative antimicrobial treatment regimes for burn and other wounds.

The potential to successfully treat infecting and colonising organisms without antibiotics is an exciting development that warrants further study.

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