Journal of Antimicrobial Chemotherapy (1994) **33**, 619–624

Sensitization of *Staphylococcus aureus* to killing by low-power laser light

Michael Wilson and Jonathan Pratten

Department of Microbiology, Institute of Dental Surgery, University of London, 256 Grays Inn Road, London WC1X 8LD, UK

Introduction

Staphylococcus aureus is the aetiological agent of a wide range of infections, both nosocomial and community-acquired, and topically-applied antimicrobials are often used for their prevention, control and treatment; for example, isopropanol/hexachlorophane is applied to the umbilicus of neonates to prevent colonization (Watkinson & Dyas, 1992), mupirocin is used to eliminate nasal carriage (Casewell & Hill, 1986) and fusidic acid is used to treat skin infections (Greenwood, 1989). However, the development of resistance to many of these topical agents has been a cause for concern (Grosserode & Wenzel, 1991) and emphasizes the need to identify new techniques to eradicate *S. aureus* from infected wounds and carriage sites.

Malignant mammalian cells are killed by exposure to low dosages of laser light when they have been pre-treated with a photosensitizing agent, and this is the basis for photodynamic therapy of tumours (Dougherty, Potter & Bellnier, 1990). A number of studies have demonstrated that bacteria can also be sensitized to killing by low-power laser light which, itself, is not bactericidal (MacMillan, Maxwell & Chichester, 1966; Wilson, 1993); this antimicrobial activity is thought to be mediated by singlet oxygen and free radicals generated by the photoactivated sensitizer (Spikes & Jori, 1987; Malik, Hanania & Nitzan, 1990). The purpose of this investigation was to determine whether this strategy could be used to kill *S. aureus in vitro*.

Materials and methods

Laser and photosensitizer

The laser used in the investigation was a gallium aluminium arsenide (GaAs) diode laser (Omega Universal Technologies Ltd, London, UK) which had a power output of 11 mW and emitted light with a wavelength of 660 nm as an uncollimated beam. Bacterial suspensions were irradiated at a distance of 6 mm so that the diameter of the beam at the surface of the suspension was 9 mm. The photosensitizer used in conjunction with the laser was aluminium disulphonated phthalocyanine ($AlPcS_2$) which was kindly provided by Prof. D. Phillips (Chemistry Department, Imperial College, London).

0305-7453/94/030619+06 $08.00/0

Determination of the effect of the $AlPcS_2$ concentration on lethal photosensitization

The bacterial strain used in the study, *S. aureus* NCTC 6571, was maintained by weekly subculture on nutrient agar (Oxoid Ltd, Basingstoke, UK). For experimental purposes, the organism was grown aerobically in nutrient broth at 37°C for 16 h. It was harvested by centrifugation and resuspended in an equal volume of 0·85% (w/v) saline. Aliquots (100 μL) of the suspension (containing *c.* 6×10^8 cfu) were transferred to the wells of 96-well, round-bottomed microtitre plates (Sterilin Ltd, Stone, UK) and an equal volume of a filter-sterilized solution of $AlPcS_2$ in 0·85% (w/v) saline was added to each well to give final concentrations ranging from 12·5 to 50 mg/L. A 4-mm magnetic stirrer bar was inserted into each well, the plates were placed on a magnetic stirrer and duplicate wells were exposed to laser light for 60 sec. Control wells containing the bacterial suspension and saline in place of the sensitizer were treated in an identical manner to determine the effect of irradiation alone on bacterial viability. In order to evaluate the antimicrobial activity of the sensitizer itself, solutions of $AlPcS_2$ in saline were added to duplicate wells containing the bacterial suspension (giving final concentrations of between 12·5 and 50 mg/L); these wells were not exposed to laser light. Two additional wells which contained the bacterial suspension and saline, in place of $AlPcS_2$, and which were not irradiated (untreated controls) were also included. Following irradiation of the appropriate wells, serial ten-fold dilutions of the contents of each well were prepared in sterile nutrient broth (Oxoid Ltd) and duplicate 50-μL aliquots were spread on to the surfaces of Wilkins-Chalgren (Oxoid Ltd) blood agar plates. After overnight incubation at 37°C, the numbers of colonies were counted and the means ($\pm$S.D.) calculated.

Effect of laser light exposure time on lethal photosensitization

The effect of varying the light exposure time on the viability of *S. aureus* was determined as described above except that the sensitizer concentration was kept constant at 12·5 mg/L while duplicate wells were exposed to laser light for periods varying from 30 to 120 sec.

Lethal sensitization of a methicillin-resistant strain of S. aureus

The susceptibility of a methicillin-resistant strain of *S. aureus* (NCTC 11939) to lethal photosensitization was determined as described above with $AlPcS_2$ at a concentration of 12·5 mg/L and laser light exposure times of 60 and 120 sec.

Statistical analysis

Student's *t*-test was used to compare the numbers of viable bacteria following treatment with the sensitizer, laser light or both with the number of organisms in the untreated control suspension; $P < 0{\cdot}05$ was considered statistically significant. The bactericidal activities achieved with the different concentrations of $AlPcS_2$ were compared by the Kruskal-Wallis one-way analysis of variance for non-parametric data.

Results

Exposure of *S. aureus* to light from the GaAs laser for 60 sec (energy dosage, 660 mJ; energy density, 1·0 J/cm^2) in the presence of $AlPcS_2$ at concentrations of 12·5, 25 and

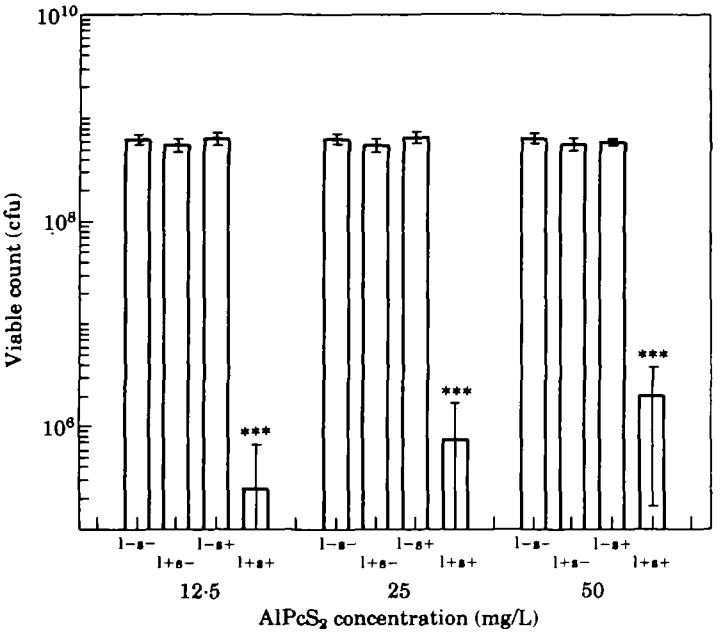

Figure 1. The effect of varying the concentration of $AlPcS_2$ on the viability of a methicillin-susceptible strain of *S. aureus* after exposure to light from a gallium aluminium arsenide laser for 60 sec. Suspensions exposed to neither sensitizer nor laser (untreated control) (l−s−); exposure to laser light in the absence of sensitizer (l+s−); exposure to sensitizer in the absence of laser light (l−s+); and exposure to laser light in the presence of sensitizer (l+s+). The data are the means (±S.D.) of four determinations; *** denotes $P < 0{\cdot}05$ compared with the untreated control.

50 mg/L resulted in 3·4 $\log_{10}$ (*c.* 99·9%) 2·9 $\log_{10}$ (*c.* 99·9%) and 2·5 $\log_{10}$ (*c.* 99%) reductions in the viable counts respectively ($P < 0{\cdot}05$ compared with the untreated control but not significantly different when compared with each other) (Figure 1). In the absence of laser light, significant decreases in the numbers of viable bacteria were not observed following treatment with $AlPcS_2$ at any of the concentrations used. Nor did irradiation alone have a significant effect on bacterial viability.

The effect of varying the exposure time to the GaAs laser on the viability of *S. aureus* in the presence of $AlPcS_2$ at a concentration of 12·5 mg/L is shown in Figure 2. Irradiation for 30 sec (energy dosage, 330 mJ; energy density, 0·5 J/cm^2) caused a 1·6 $\log_{10}$ (> 90%) reduction in the viable count ($P < 0{\cdot}05$ compared with the untreated control suspension). Prolonging the exposure time produced even greater bactericidal activity i.e. a 3·4 $\log_{10}$ (*c.* 99·9%) reduction in the number of cfu at 60 sec (energy dosage, 660 mJ; energy density, 1·0 J/cm^2) and a 4·0 $\log_{10}$ (99·99%) reduction at 120 sec (energy dosage, 1320 mJ; energy density, 2·0 J/cm^2); no bactericidal activity was observed when the suspension was exposed to either the sensitizer or laser light alone.

Irradiation of the methicillin-resistant strain of *S. aureus* with the GaAs laser for 120 sec in the presence of $AlPcS_2$ (12·5 mg/L) resulted in a 3·3 $\log_{10}$ (*c.* 99·9%) reduction in the viable count (Figure 3).

Discussion

The present study has demonstrated that more than 99% of a *S. aureus* suspension can be killed by short-term exposure to light from an 11-mW GaAs laser with $AlPcS_2$ as an

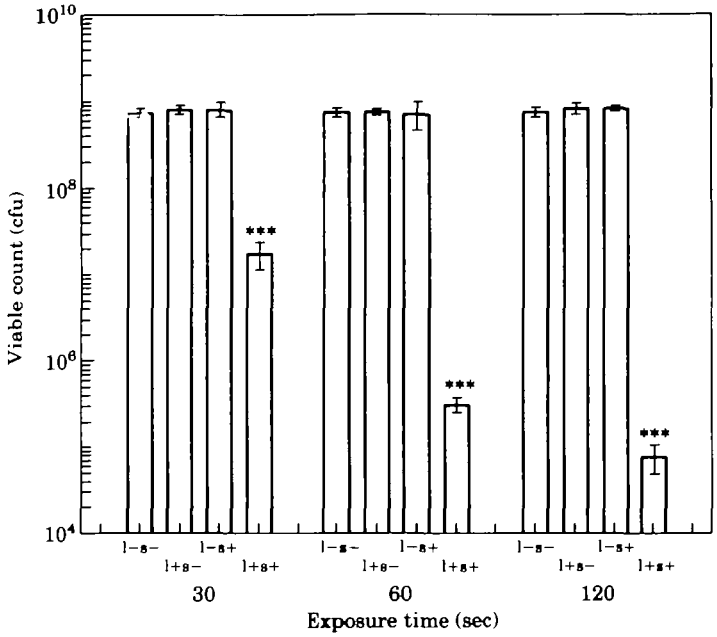

Figure 2. The effect of varying the laser light exposure time on *S. aureus* viability ($AlPcS_2$ concentration, 12·5 mg/L). The data are the means (±S.D.) of four determinations; *** denotes $P < 0{\cdot}05$ compared with the untreated control.

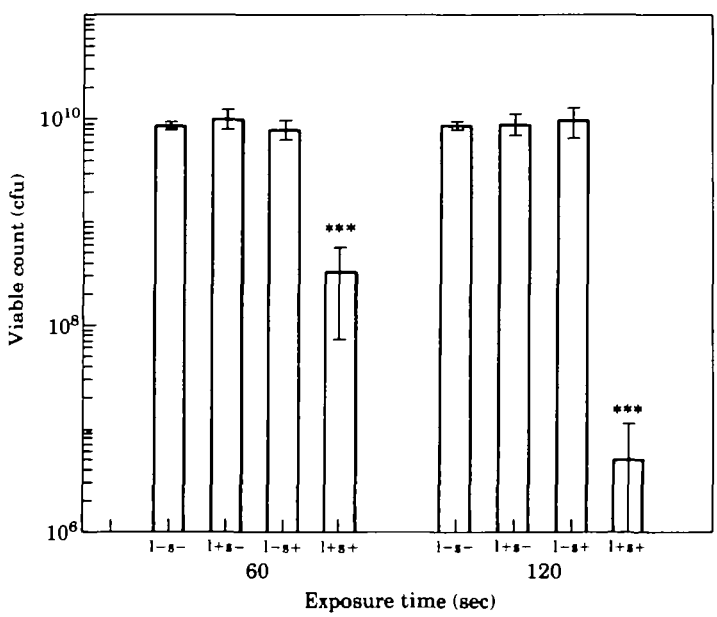

Figure 3. The susceptibility of a methicillin-resistant strain of *S. aureus* to lethal photosensitization ($AlPcS_2$ concentration, 12·5 mg/L). The data are the means (±S.D.) of four determinations; *** denotes $P < 0{\cdot}05$ compared with the untreated control.

exogenous photosensitizer. The bactericidal activities achieved in the presence of $AlPcS_2$ at a concentration of 12·5 mg/L were equivalent to approximately 3 $\log_{10}$ and 4 $\log_{10}$ reductions in the numbers of cfu after exposure for 60 and 120 sec, respectively. No significant activity was detected when the bacteria were exposed to either the sensitizer or the laser light alone; the latter observation is consistent with the absence of an endogenous photosensitizer which might have acted in conjunction with light with a wavelength of 660 nm at the energy densities used in the study.

Although $AlPcS_2$ has previously been shown to effectively sensitize both *Helicobacter pylori* and *Porphyromonas gingivalis* to killing by low-power laser light (Bedwell *et al.*, 1990; Wilson, Dobson & Sarkar, 1993), this appears to be the first report in which this agent has been used to induce the lethal photosensitization of *S. aureus*. Earlier studies with this organism employed predominantly haematoporphyrins as sensitizers in combination with a white light source (Malik *et al.*, 1990). However, while considerable cidal activity was obtained with this sensitizer/light combination, the exposure times and photosensitizer concentrations required to achieve this effect were substantially greater than those used in the present study. For example, Venezio *et al.* (1985) reported a 99·9% kill (of an unspecified number of bacteria) after exposure to light from a 300-W projector lamp for 20 min in the presence of an haematoporphyrin derivative. Similarly, following irradiation with light from four 250-W tungsten bulbs, Bertoloni *et al.* (1983) demonstrated a 5-log reduction in the number of viable organisms in a suspension of haematoporphyrin-sensitized *S. aureus* which initially contained 10^{12} cfu/L. However, the irradiation time employed was 10 min and the concentration of the photosensitizer was 200 mg/L. The greater energy densities achieved with laser light allow much shorter exposure times to be used and this would have obvious advantages if the technique was to be adapted for the purpose of eradicating *S. aureus* from an infected or a colonized site.

Whilst the results of this study demonstrate the efficacy of a GaAs laser light in terms of killing $AlPcS_2$-sensitized *S. aureus*, a number of aspects of the technique need to be investigated further before it can be evaluated in the clinical setting. Firstly, concerns that host cells (particularly those of the epithelium) might also be killed during treatment must be addressed. It has previously been shown that following the systemic administration of $AlPcS_2$, irradiation of rabbit gingiva with light from a copper vapour-pumped dye laser was associated with the appearance of small ulcers which healed within 2 weeks (Meyer, Speight & Bown, 1991). In that study, however, the total energy dosage (20 J) and density (64 KJ/cm^2) were approximately 15-fold and 32,000-fold greater, respectively, than those determined in the present study to be necessary for the lethal photosensitization of *S. aureus*. None the less, should in-vitro investigations reveal that injury to mammalian cells occurs at the energy dosages and photosensitizer concentrations required to kill *S. aureus*, then a strategy for avoiding this adverse effect would need to be developed. This might be achieved by linking a photosensitizer to an anti-staphylococcal antibody, thereby ensuring that the photosensitizer is taken up exclusively by the bacterium. Specific targeting of bacteria in this way has already been demonstrated with *Pseudomonas aeruginosa* and has facilitated the selective killing of this organism in the presence of *S. aureus* (Friedberg *et al.*, 1991). A second issue requiring additional consideration concerns the potential effect of the local environment on lethal photosensitization. Host secretions such as serum, may interfere with the binding of the photosensitizer to the target organism, consequently reducing the extent of photosensitization.

The results of this investigation have shown that two strains of *S. aureus*, one of which was methicillin-resistant, are killed by short-term exposure to low dosages of laser light after they have been treated with an appropriate photosensitizer. If similar activity can be demonstrated *in vivo*, lethal photosensitization may offer a simple and effective means of eradicating this pathogen from wounds, burns and carriage sites. Such a technique would have the following advantages: an extremely rapid bactericidal effect; antimicrobial activity would be confined to those parts of the body exposed to both sensitizer and laser light, thereby minimizing disruption of the microflora at sites other than those which have been targeted; and the unlikelihood of the development of resistance as this would have to entail the acquisition of mechanisms which protect the bacterium against the lethal effects of free radicals and singlet oxygen.

References

Bedwell, J., Holton, J., Vaira, D., MacRobert, A. J. & Bown, S. G. (1990). *In vitro* killing of *Helicobacter pylori* with photodynamic therapy. *Lancet* **335,** 1287.

Bertoloni, G., Dall'Acqua, M., Vazzoler, M., Salvato, B. & Jori, G. (1983). Bacterial and yeast cells as models for studying haematoporphyrin photosensitization. In *Porphyrins in Tumour Phototherapy* (Andreoni, A. & Cubeddu, R., Eds), pp. 177–83. Plenum, New York.

Casewell, M. W. & Hill, R. (1986). Elimination of nasal carriage of *Staphylococcus aureus* with mupirocin (pseudomonic acid)—a controlled trial. *Journal of Antimicrobial Chemotherapy* **17,** 365–72.

Dougherty, T. J., Potter, W. R. & Bellnier, D. (1990). Photodynamic therapy for the treatment of cancer: current status and advances. In *Photodynamic Therapy of Neoplastic Disease,* Vol. 1 (Kessel, D., Ed.), pp. 1–19. CRC Press, Boca Raton.

Friedberg, J. S., Tompkins, R. G., Rakestraw, S. L., Warren, S. W., Fischman, A. J. & Yarmush, M. L. (1991). Antibody-targeted photolysis. Bacteriocidal effects of Sn (IV) chlorine$_6$-dextran-monoclonal antibody conjugates. *Annals of the New York Academy of Sciences* **618,** 383–93.

Greenwood, D. (1989). *Antimicrobial Chemotherapy*, 2nd edn. Oxford University Press, Oxford.

Grosserode, M. H. & Wenzel, R. P. (1991). The continuing importance of staphylococci as major hospital pathogens. *Journal of Hospital Infection* **19,** 3–17.

MacMillan, J. D., Maxwell, W. A. & Chichester, C. O. (1966). Lethal photosensitization of microorganisms with light from a continuous-wave gas laser. *Photochemistry and Photobiology* **5,** 555–65.

Malik, Z., Hanania, J. & Nitzan, Y. (1990). Bactericidal effects of photoactivated porphyrins—an alternative approach to antimicrobial drugs. *Journal of Photochemistry and Photobiology* **5,** 281–93.

Meyer, M., Speight, P. & Brown, S. G. (1991). A study of the effects of photodynamic therapy on the normal tissues of the rabbit jaw. *British Journal of Cancer* **64,** 1093–7.

Spikes, J. D. & Jori, G. (1987). Photodynamic therapy of tumours and other diseases using porphyrins. *Lasers in Medical Science* **2,** 3–15.

Venezio, F. R., DiVincenzo, C., Sherman, R., Reichman, M., Origitano, T. C., Thompson, K. *et al.* (1985). Bactericidal effects of photoradiation therapy with haematoporphyrin derivative. *Journal of Infectious Diseases* **151,** 166–9.

Watkinson, M. & Dyas, A. (1992). *Staphylococcus aureus* still colonizes the untreated neonatal umbilicus. *Journal of Hospital Infection* **21,** 131–6.

Wilson, M. (1993) Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. *Journal of Applied Bacteriology* **75,** 299–306.

Wilson, M., Dobson, J. & Sarkar, S. (1993). Sensitisation of periodontopathogenic bacteria to killing by light from a low-power laser. *Oral Microbiology and Immunology* **8,** 182–7.

(Received 28 July 1993; accepted 29 October 1993)