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Selective lethal photosensitization of methicillin-resistant *Staphylococcus aureus* using an IgG–tin (IV) chlorin e6 conjugate

Michelle L. Embleton[1,2], Sean P. Nair[2], Barry D. Cookson[3] and Michael Wilson[1]*

[1]Department of Microbiology and [2]Cellular Microbiology Research Group, Eastman Dental Institute for Oral Health Care Sciences, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK; [3]Laboratory of Hospital Infection, Central Public Health Laboratory, Colindale, London

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Objectives. **The growing resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) to conventional antimicrobial agents necessitates the development of alternative approaches to preventing and treating infections. One such approach is photodynamic therapy, whereby target cells are treated with light-activated drugs (photosensitizers). This investigation aimed to determine whether the ability of MRSA to express the IgG-binding protein, protein A, could be exploited to enable selective lethal photosensitization of the organism with a photosensitizer [tin (IV) chlorin e6; SnCe6] linked to IgG.**

Methods. **Various strains of MRSA were exposed to light from a helium/neon laser in the presence of an IgG–SnCe6 conjugate and the survivors enumerated by viable counting. Controls consisted of suspensions irradiated in the presence or absence of the conjugate and suspensions kept in the dark in the presence of the conjugate. Similar experiments were also carried out using the unconjugated photosensitizer. The experiments were repeated using a suspension consisting of both EMRSA-16 and *Streptococcus sanguis*.**

Results. **EMRSA-16 was killed by IgG–SnCe6 and SnCe6 in a light-dose- and photosensitizer-dependent manner. Greater kills were achieved with the IgG–SnCe6 than with the unconjugated SnCe6 using the same light energy dose and photosensitizer concentration. Furthermore, the IgG–SnCe6 conjugate, but not SnCe6, was able to kill EMRSA-16 selectively in a suspension that also contained *S. sanguis* without any reduction in the viable count of the latter.**

Conclusion. **These results demonstrate that selective lethal photosensitization of MRSA can be achieved using an IgG–tin (IV) chlorin e6 conjugate. The effectiveness of killing was dependent, in part, on the particular MRSA strain used, with the clinically important EMRSA-16 strain being the most susceptible.**

Keywords: MRSA, tin (IV) chlorin e6, IgG-binding protein A

Introduction

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are one of the major causes of hospital-acquired infection throughout the world, causing significant infections and morbidity in many patients.[1] Such infections can be difficult to treat and infected patients may be colonized for many months, requiring lengthy hospital stays. This places strains on financial and human resources, which are often already overstretched. Since the first report of resistance to methicillin in 1961,[2] these organisms have demonstrated resistance to a wide variety of antimicrobials, leaving some forms of multidrug-resistant MRSA susceptible only to glycopeptide antimicrobials, e.g. vancomycin and teicoplanin. However, tolerance of MRSA even to these drugs has been reported.[3–5] Therefore, there is a pressing need to develop alternative strategies for the treatment and prevention of MRSA infections.

*Corresponding author: Tel: +44-207-915-1231; Fax: +44-207-915-1127; E-mail: M.Wilson@eastman.ucl.ac.uk

One possible approach is photodynamic therapy (PDT): the use of light-activated antimicrobial agents to achieve lethal photosensitization of the infecting organism.[6–8] Basically, this involves treating the microbe with a light-activated chemical, termed a photosensitizer. Alone, a photosensitizer generally has no, or negligible, bactericidal activity. However, upon exposure to light of a wavelength absorbed by the photosensitizer, the photosensitizer undergoes a transition from a lower-energy 'ground state' to a higher-energy 'triplet state'. The triplet state photosensitizer can react with solvent and substrate molecules to produce free radicals and radical ions, or with molecular oxygen resulting in the generation of singlet oxygen.[9] Such reactive species, in particular singlet oxygen, can damage plasma membranes and DNA, leading to cell death.[10,11] The killing of bacteria in this way has been demonstrated with a wide variety of Gram-positive and Gram-negative organisms, e.g. *S. aureus*, including MRSA,[10,12–15] streptococcal species,[16,17] *Escherichia coli*[16] and *Porphyromonas gingivalis*.[11,18]

Photodynamic therapy could be a useful and simple way of treating and controlling MRSA infections and would have two major advantages over conventional antiseptics and antimicrobials. First, as neither of the two components of the system (light and sensitizer) is inherently bactericidal, the antibacterial effect would be limited to light-irradiated regions of the sensitizer-treated area, avoiding the disruption of the normal microflora at sites other than those being targeted. Secondly, and perhaps most importantly, the development of resistance to photochemically induced killing, which is mediated predominantly by singlet oxygen, would be unlikely.

Nevertheless, there are potential problems associated with PDT of infectious diseases, one of which is a lack of specificity. The photosensitizer may bind to, or be taken up by, other cells e.g. commensal microorganisms or host cells. Since subsequent irradiation of such cells could lead to their destruction, it is desirable to direct the photodynamic activity to the target cell. This could be achieved by linking the photosensitizing molecule to an antibody against the surface of the target organism. Such specific targeting is aided by the fact that the main bactericidal species, singlet oxygen, generated by the irradiation of the photosensitizer, has a very short lifetime and a limited diffusion distance of 100 nm. The antibody-targeting approach has been used successfully to target specifically different photosensitizers to a variety of bacteria: tin (IV) chlorin e6 to *Pseudomonas aeruginosa*,[19,20] toluidine blue O (TBO) to *Porphyromonas gingivalis*[21] and bacteriochlorophyll to *S. aureus*.[22]

In this study, we aimed to direct a suitable photosensitizer to *S. aureus*, including MRSA, while retaining the photodynamic activity of the molecule. Previous studies have shown that *S. aureus* is sensitive to lethal photosensitization using a variety of photosensitizers, e.g. porphyrins, thiazin dyes, phthalocyanines and chlorins.[10,12–14,17,23,24] Since the HeNe laser we used in this study emits red light with a wavelength of 632.8 nm, it was essential to choose a photosensitizer that would produce high yields of singlet oxygen when exposed to such light. In addition, it was necessary to select a photosensitizer with side chains that would enable conjugation to targeting molecules. Tin (IV) chlorin e6 (SnCe6) fits both of these criteria; it absorbs light at 632 nm and has an improved yield of singlet oxygen over related chlorins,[25] while possessing reactive carboxyl groups on side chains outside the polycyclic core. The structure of SnCe6 is shown in Figure 1.

To direct SnCe6 to the surface of *S. aureus*, we chose to target the *S. aureus* cell wall protein, protein A. This protein binds to many isotypes of IgG through the Fc region of the immunoglobulin.[26] Hence, by conjugating SnCe6 to IgG, the photosensitizer should be directed to the surface of *S. aureus*. Although some strains of *S. aureus* are low or non-producers of protein A, we have selected this as a target due to the ready availability of IgG compared with other (monoclonal) antibodies against the surface of *S. aureus*. We aimed not only to determine the effectiveness of targeted SnCe6 versus free SnCe6, but also to see if a conjugate of IgG and SnCe6 would specifically target MRSA in a cell suspension containing a mixed population of Gram-positive bacteria.

Materials and methods

Bacterial strains

The organisms used in this investigation were the prototypic UK epidemic MRSA, EMRSA-1 (NCTC 11939); the three currently prevalent EMRSA, EMRSA-3 (NCTC 13130), EMRSA-15 (NCTC 13142) and EMRSA-16 (NCTC 13143); a methicillin-susceptible strain of *S. aureus* (NCTC 6571); and *Streptococcus sanguis* (NCTC 7863). Strains were maintained by weekly subculture on Columbia Agar (Oxoid Ltd, Basingstoke, UK) supplemented with 5% (v/v) horse blood. For experimental purposes, a few colonies were inoculated

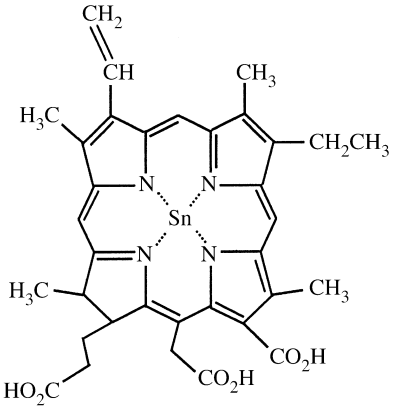

Figure 1. Structure of tin (IV) chlorin e6.

into tryptone soya broth (Oxoid): *S. aureus* strains were grown aerobically for 16 h at 37°C, *S. sanguis* was grown for 16 h at 37°C in a 5% CO_2 cabinet. Cells were then harvested by centrifugation and resuspended in sterile phosphate-buffered saline (PBS) to an OD_{600} of 0.4. For growth-phase experiments, 100 mL of tryptone soya broth was inoculated with 0.25 mL of an overnight culture and growth at 37°C monitored by measuring OD_{600}. Cells were harvested at lag (OD_{600} 0.05), exponential (OD_{600} 0.55) and stationary phase (OD_{600} 2.3).

Laser and photosensitizer

The laser used in this study was a Model 127 Stabilite helium–neon laser (Spectra Physics, Mountain View, CA, USA), with a measured output of 35 mW, which emits light in a collimated beam (diameter 1.25 mm) with a wavelength of 632.8 nm. The photosensitizer, tin (IV) chlorin e6 (SnCe6) was purchased from Frontier Scientific (Lancashire, UK).

Preparation of the IgG–SnCe6 conjugate

SnCe6 was conjugated to IgG exploiting the carboxyl side chains on the SnCe6 molecule (Figure 1), and amino groups in IgG, adapting the method described in Bhatti *et al.*[21] Basically, 2 mg of SnCe6 was dissolved in 1.5 mL conjugation buffer [0.1 M 2-(*n*-morpholino)ethanesulphonic acid pH 6.0, 0.5 M NaCl] along with 2 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) (Sigma, Poole, UK) and 5 mM sulpho-*N*-hydroxysuccinimide (S-NHS) (Pierce, Cheshire, UK). EDAC reacts with the carboxyl groups of SnCe6 in the presence of S-NHS to form a reactive ester. After 4 h at room temperature, the reaction was quenched by the addition of β-mercaptoethanol to 20 mM. The treated SnCe6 (~1.5 mL) was then added to 2 mL of a 4 mg/mL solution of rabbit IgG (Sigma) in PBS and the pH adjusted to 7.6 to allow the amine-reactive intermediate to react with amine groups of the antibody. Following a 16–20 h incubation at room temperature, the conjugation reaction was terminated by the addition of ethanolamine to 20 mM. The resulting IgG–SnCe6 conjugate was purified from any unconjugated SnCe6 by affinity chromatography using Protein A Hi-Trap columns (Amersham Pharmacia Biotech, Buckinghamshire, UK). The purified IgG–SnCe6 was dialysed in PBS. The amount of SnCe6 in the conjugate was measured by absorbance at 633 nm and IgG by absorbance at 280 nm. The molar ratio of IgG:SnCe6 varied from batch to batch, but was, on average, ~2:1. The concentration of the conjugate is expressed in terms of the concentration of SnCe6 in the conjugate.

Lethal photosensitization

Aliquots (100 μL) of a suspension of *S. aureus* (containing between 1×10^9 and 1×10^{10} cfu/mL) in sterile PBS, were placed into a 96-well microtitre plate. To duplicate wells, 100 μL of either SnCe6 or IgG–SnCe6 was added. Unless otherwise stated, the samples treated with SnCe6 were pre-incubated for 1 min at room temperature. Samples treated with IgG–SnCe6 were pre-incubated for 15 min at room temperature to allow the conjugate to bind to the cells. Samples were then exposed to a measured amount of laser light as stated, where a light dose of 21 J/cm^2 corresponds to a 5 min exposure, and stirred continuously during this time. Control wells were neither sensitized nor exposed to the light source. Following exposure to light, 100 μL aliquots were taken and added to 900 μL tryptone soya broth (Oxoid). Serial dilutions were made, and 50 μL aliquots (in duplicate) from each dilution were plated on to Columbia agar, and grown overnight at 37°C to enumerate survivors. For targeting experiments using *S. sanguis*, either 100 μL of *S. sanguis* or 50 μL of both EMRSA and *S. sanguis* were placed into the wells and the experiments repeated as before. Serial dilutions of light-treated samples were plated on to tryptone soya agar (Oxoid) and grown at 37°C in a 5% CO_2 cabinet for 16 h to enumerate *S. aureus* survivors and for 40 h to enumerate *S. sanguis* survivors. The two strains were distinguishable on the basis of colony size and appearance.

Results

SnCe6 is an effective photosensitizer of S. aureus

When *S. aureus* NCTC 6571 was treated with 25 μg/mL SnCe6 and exposed to 8.4 J/cm^2 of red light from the HeNe laser (noted as L+S+ for exposure to light and exposure to sensitizer), a 2.5 $\log_{10}$ reduction in the viable count was achieved when compared with a control sample that was neither sensitized nor irradiated (noted as L–S–) (Figure 2). This amounted to 99.7% of the cells being killed (6.75×10^9 cfu/mL). Cells treated with SnCe6 but not irradiated

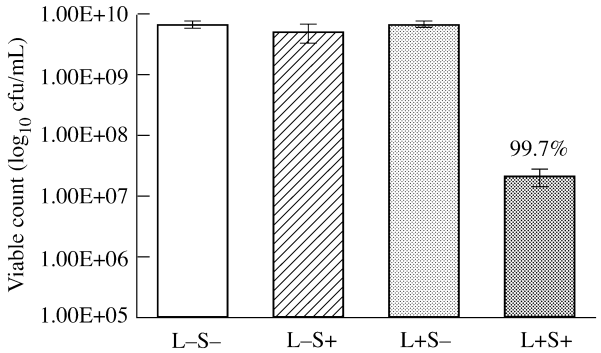

Figure 2. Lethal photosensitization of *S. aureus* NCTC 6571 with 25 μg/mL SnCe6. An equal volume of either 50 μg/mL SnCe6 (L–S+ and L+S+) or PBS (L+S– and L–S–) was added to *S. aureus* and samples were either sensitized by exposure to 8.4 J/cm^2 HeNe laser light (L+S– and L+S+) or kept in the dark (L–S+ and L–S–).

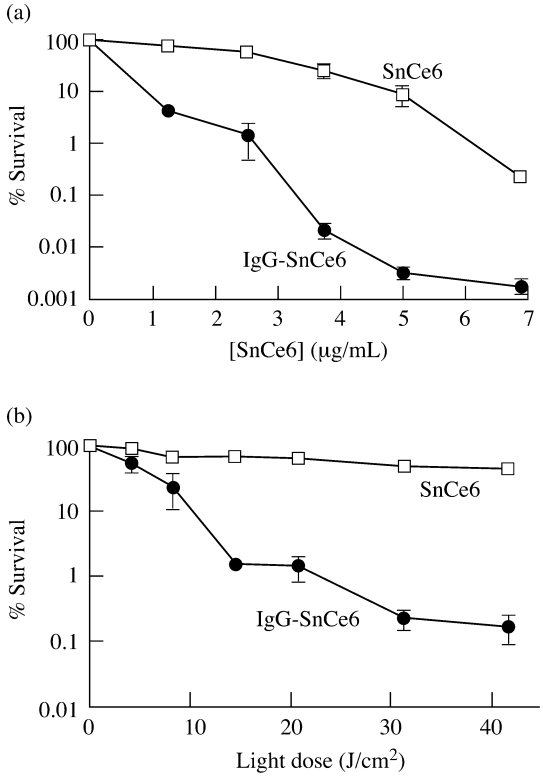

Figure 3. Lethal photosensitization of EMRSA-16 with SnCe6 and IgG–SnCe6. (a) SnCe6 (open squares) or IgG–SnCe6 (filled circles) was added to EMRSA-16 to the concentrations indicated and exposed to 21 J/cm^2 laser light. (b) SnCe6 (open squares) or IgG–SnCe6 (filled circles) was added to EMRSA-16 to a final concentration of 3 μg/mL and samples were exposed to various doses of laser light, as indicated.

(L–S+), or those not treated with SnCe6 but exposed to red light (L+S–) did not show a significant reduction in viability (Figure 2).

Effectiveness of SnCe6 versus IgG–SnCe6 against EMRSA-16

To determine the relative effectiveness of IgG–SnCe6 and SnCe6 as photosensitizers in the lethal photosensitization of MRSA, EMRSA-16 was subjected to varying concentrations of photosensitizer and different light doses (Figure 3). Figure 3(a) shows that the proportion of survivors decreased as the concentration of SnCe6 or IgG–SnCe6 conjugate was increased and the IgG–SnCe6 conjugate killed more cells than the equivalent concentrations of free SnCe6, using the same light energy dose of 21 J/cm^2 (5 min exposure). At the highest concentration of IgG–SnCe6 used, 6.9 μg/mL, over 99.99% of the bacteria were killed (a 4.9 $\log_{10}$ reduction in the viable count). Figure 3(b) shows the proportion of EMRSA-16 surviving following treatment with SnCe6 or IgG–SnCe6 at 3 μg/mL, exposed to different doses of light from the HeNe laser. The higher the light dose, the lower the percentage of EMRSA-16 surviving and, again, IgG–SnCe6 produced higher kills than SnCe6 alone. From these graphs (Figure 3a and b) it can be seen that IgG–SnCe6 was much more effective than unconjugated SnCe6 at killing EMRSA-16.

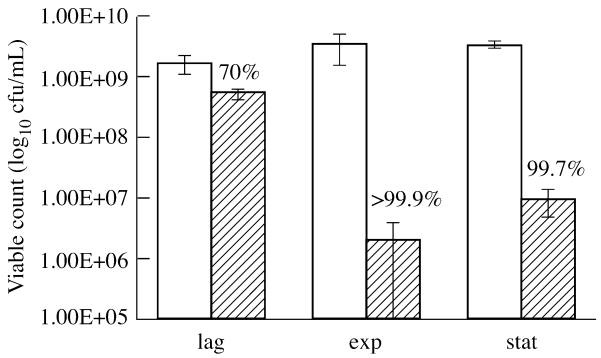

Figure 4. The effect of different growth phases on the sensitivity of EMRSA-16 to killing by IgG–SnCe6 and laser light. To cells of lag, mid-exponential (exp) and stationary phase (stat), 4.25 μg/mL IgG–SnCe6 was added and samples exposed to 21 J/cm^2 of laser light (grey bars). Control cells (white bars) were neither treated with photosensitizer nor exposed to laser light. The percentage of cells killed is shown above the corresponding bars.

To ensure that the killing of EMRSA-16 by IgG–SnCe6 in the presence of laser light was due to the conjugated SnCe6 rather than to IgG itself, control samples of EMRSA-16 treated with unconjugated IgG at a concentration equal to that in the conjugate, in the presence and absence of SnCe6 at 4.25 μg/mL, were exposed to 21 J/cm^2 light. No reduction in viability was observed with IgG alone, whereas the combination of 4.25 μg/mL SnCe6 and IgG gave similar levels of kills to that seen with 4.25 μg/mL SnCe6 alone. Thus, the killing of MRSA by IgG–SnCe6 was attributable to photodynamic activity, and not to the presence of IgG.

Lethal photosensitization of EMRSA-16 at different growth phases

The effect of the growth phase of EMRSA-16 on lethal photosensitization was tested using the IgG–SnCe6 conjugate (Figure 4). The viability of exponential and stationary phase bacteria were both dramatically reduced by the combination of IgG–SnCe6 and red light, whereas bacteria in the lag phase were less susceptible. As the expression of protein A is regulated in a growth phase-dependent manner,[27,28] the lag phase bacteria may have less protein A on the cell surface to bind the IgG–SnCe6 conjugate. Suspensions of lag, exponential and stationary phase bacteria were also subjected to lethal photosensitization with free (unconjugated) SnCe6 at 25 μg/mL in combination with a light dose of 8.4 J/cm^2. Under these conditions the bacteria in the lag phase were again the least susceptible to lethal photosensitization; 60% of lag phase, 91% of exponential phase and 97% of stationary phase bacteria were killed.

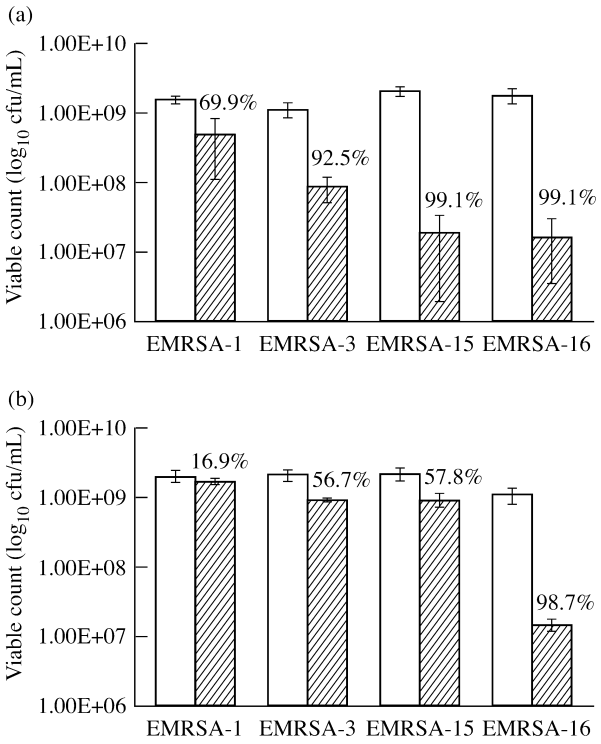

Figure 5. Killing of MRSA strains by lethal photosensitization. SnCe6 (a) or IgG–SnCe6 (b) was added to MRSA strains to concentrations of 25 μg/mL and 4.25 μg/mL, respectively, and exposed to 21 J/cm^2 laser light (grey bars). In both (a) and (b), control samples (white bars) were neither treated with photosensitizer nor exposed to laser light. The percentage kill for each strain is shown above the corresponding grey bar.

Susceptibility of different EMRSA strains

When EMRSA strains 1, 3, 15 and 16 were subjected to lethal photosensitization using SnCe6 and laser light (Figure 5a), the kills attained using SnCe6 at 25 μg/mL and a light dose of 21 J/cm^2 varied with the strain used. Figure 5(a) shows that EMRSA-1 was the least susceptible, with <70% of the cells being killed. In comparison, the viable counts of EMRSA-15 and EMRSA-16 were reduced by >99% (Figure 5a). The effectiveness of lethal photosensitization using IgG–SnCe6 was also found to be strain dependent (Figure 5b): 98.7% of EMRSA-16 were killed compared with only 16.9% of EMRSA-1 using 21 J/cm^2 light and 4.25 μg/mL IgG–SnCe6.

Targeting of IgG–SnCe6 to EMRSA-16

The targeting of the IgG–SnCe6 conjugate towards MRSA was tested by comparing the kills of EMRSA-16 with that of another Gram-positive bacterium, *S. sanguis*, in single and mixed cultures (Figure 6). When *S. sanguis* was treated with SnCe6 and exposed to HeNe laser light under similar conditions to those used for EMRSA-16 (Figure 5a), the viable count was reduced by 99.8% (3 $\log_{10}$ reduction). In contrast, when *S. sanguis* was treated with IgG–SnCe6 under compar-

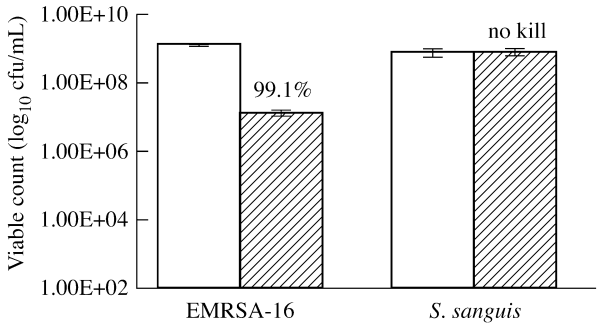

Figure 6. Targeting of IgG–SnCe6 to EMRSA-16 in a mixed population of *S. sanguis* and EMRSA-16. To a cell suspension containing EMRSA-16 and *S. sanguis*, IgG–SnCe6 was added to 4.25 μg/mL and cells exposed to 21 J/cm^2 laser light (grey bars). Control cells (white bars) were neither treated with photosensitizer nor exposed to laser. The proportion of each species killed is shown above the corresponding grey bar.

able conditions to those used for EMRSA-16 (Figure 5b), only 16.3% kill was achieved. Therefore, although SnCe6 is an effective photosensitizer of *S. sanguis*, the IgG–SnCe6 conjugate is much less effective against *S. sanguis* compared with EMRSA-16. When the IgG–SnCe6 conjugate was added to a mixed culture containing approximately equal proportions of EMRSA-16 and *S. sanguis*, and irradiated, 99.1% of the EMRSA-16 were killed, whereas no reduction in the number of viable *S. sanguis* cells was observed (Figure 6).

Discussion

The purpose of this investigation was to target the photosensitizer SnCe6 to MRSA to enable efficient and selective killing of the organism. We compared the effectiveness of an IgG–SnCe6 conjugate, which exploited the IgG-binding property of protein A found on the surface of *S. aureus*, with its undirected counterpart, SnCe6. Although lethal photosensitization using both SnCe6 and SnCe6 conjugates was dose dependent with respect to both sensitizer concentration and light energy, IgG–SnCe6 was much more effective than SnCe6 at killing EMRSA-16. For example, after exposure to 21 J/cm^2 light, a concentration of 1.25 μg/mL IgG–SnCe6 killed more than three times the number of cells in a suspension containing ~10^9 cells, than free SnCe6.

The greater efficacy and potency of the IgG–SnCe6 conjugate over free SnCe6 is likely to be due to its targeting of protein A, enabling the SnCe6 to be in closer proximity to the cells. Logically, the greater the amount of SnCe6 bound to or associated with the cells, the more singlet oxygen the cells will be exposed to. Hence, cells treated with IgG–SnCe6 may have a higher concentration of the photosensitizer at the cell surface and thus a greater exposure to singlet oxygen, than those treated with SnCe6 alone. There is evidence to suggest that photosensitizers that penetrate into the bacterial cell may

be more effective than those that are at the cell surface.[29,30] However, this does not mean that photosensitizers located at the cell surface are not efficacious, as this and other studies[11,18–21] have shown.

Although both IgG–SnCe6 and SnCe6 were able to kill EMRSA-16 when irradiated with red light, there were variations in the susceptibility of the other MRSA strains to this treatment. Using concentrations of SnCe6 (unconjugated) chosen to demonstrate differences in the susceptibility of the bacteria to this treatment, rather than to achieve 100% kill, we found that, in a suspension containing ~10^9 cells, almost all of *S. aureus* NCTC 6571, EMRSA-15 and EMRSA-16 were killed with a combination of 25 μg/mL SnCe6 and a light dose of 8.4 J/cm^2. However, <70% of EMRSA-1 were killed under these conditions. Wainwright *et al.*[23] also reported such an effect, when using thiazin and phenothiazinium dyes as photosensitizers against pathogenic strains of *S. aureus*, some of which were EMRSA strains. The authors found that although the photosensitizers were effective against all strains used, they were less effective against the MRSA strains than against *S. aureus* NCTC 6571. This could be due to differences in the composition of the cell wall or membrane, rendering certain strains less susceptible to the effects of singlet oxygen, or due to a variation in the uptake of the photosensitizers between the strains, or possibly even both. In contrast, another such study, using aluminium disulphonated phthalocyanine ($AlPcS_2$) against 16 different EMRSA strains, found that there were no marked differences in susceptibility among the strains.[12]

The MRSA strains also had varying susceptibilities to the IgG–SnCe6 conjugate; however, this was not the same as with unconjugated SnCe6. When using the conjugate, the variation between the strains may be related to the amount of protein A expressed by each strain, and its attachment to the cell wall. Indeed, different strains of *S. aureus*, whether methicillin resistant or methicillin sensitive, can vary in the amount of protein A expressed and localized to the cell surface[31] and there is some evidence to suggest that EMRSA-16 has more surface-bound protein A than EMRSA-15, EMRSA-3 and EMRSA-1.[32] This would help to explain why free SnCe6 reduced the viable counts of EMRSA-16 and EMRSA-15 by 99.1%, whereas with IgG–SnCe6, EMRSA-16 was much more susceptible to killing than EMRSA-15, with 98.7% and 57.8% kills, respectively. If EMRSA-16 produces larger amounts of cell wall bound protein A than EMRSA-15, it would be expected that a greater amount of the conjugate would bind to EMRSA-16 than EMRSA-15. Hence, upon exposure to laser light, more singlet oxygen would be produced in the vicinity of EMRSA-16, resulting in higher kills of this strain.

The expression of protein A can also go some way to explaining the growth phase-dependent lethal photosensitization of MRSA. Protein A is expressed during the exponential phase of growth and the *spa* gene is repressed by Agr and Sar in the post-exponential phase.[33,34] EMRSA-16 was most susceptible during the exponential phase, coinciding with maximal protein A expression. Cells in the lag phase of growth were the least sensitive to lethal photosensitization by IgG–SnCe6. However, this effect cannot be due to protein A alone, since cells treated with unconjugated SnCe6 also showed a greater resistance to killing when in the lag phase. Conversely, studies using $AlPcS_2$ as the sensitizer demonstrated that the killing of MRSA was not dependent on growth phase.[15] Similarly, no growth-phase effects have been observed with the photosensitizers toluidine blue and azure A (M. L. Embleton, unpublished observations). Thus, it seems likely that the effect of growth phase on the susceptibility of *S. aureus* to lethal photosensitization is a characteristic of the photosensitizer used.

A protein A-mediated targeting approach to direct the photosensitizer bacteriochlorophyll (Bchl) to the surface of *S. aureus* Cowan I was also used by Gross *et al.*[22] In these experiments, an IgG–Bchl conjugate was tested alongside a non-targeted serine–Bchl conjugate for its ability to bind to and, upon exposure to light with $\lambda > 550$ nm, kill the bacterium. The targeted IgG–Bchl conjugate was found to be much more effective than the serine–Bchl. The higher efficacy of the IgG–Bchl was explained by the exclusive positioning of the conjugate on the bacterial cell wall. However, these experiments were performed on *S. aureus* Cowan I, a strain that is naturally abundant in protein A[35,36] and, therefore, would be very able to bind the immunoconjugate. The conjugate was not tested for its effectiveness against other strains of *S. aureus*, for example low, or non-producers of protein A, nor was it tested for its specificity for *S. aureus* in a mixed culture with other bacteria.

We have not only tested our IgG–SnCe6 conjugate against different strains of *S. aureus*, but have also shown that IgG–SnCe6 was able to kill EMRSA-16 when mixed with another Gram-positive organism. Like EMRSA-16, *S. sanguis* is very susceptible to killing by a combination of SnCe6 and red light. However, unlike EMRSA-16, *S. sanguis* was not killed efficiently (16.3%) by the IgG–SnCe6 conjugate. This was to be expected, since *S. sanguis* has not been documented as having IgG-binding proteins on its surface. When IgG–SnCe6 was used with a mixed culture of EMRSA-16 and *S. sanguis*, >99% of the EMRSA-16 were killed with no reduction in the viable count of *S. sanguis*. Thus, IgG–SnCe6 is able to kill EMRSA-16 selectively.

In this investigation, we have achieved our aims by demonstrating that a photosensitizer can be targeted to an EMRSA and retain its photodynamic activity. Upon exposure to light of an appropriate wavelength, the targeted bacteria are killed, whereas untargeted microorganisms in close proximity to the target MRSA are not. The effectiveness of this IgG–SnCe6 conjugate against the clinically important EMRSA-16 strain

may make it a good candidate for treatment of topical EMRSA-16 infections, e.g. wounds and burns, ulcers, pressure sores and prosthetic or catheter biofilm-associated infections. Furthermore, it could be used to prevent the spread of EMRSA-16 by eliminating the organism from carriage sites such as the nares, throat and skin. However, this may not be a cost-effective strategy in the longer term in view of the waxing and waning of EMRSAs in the UK.

The experiments have also demonstrated that future targeting strategies may have to use cell wall targets that are more uniformly distributed. Providing the antibodies become, or are made, available, other surface proteins could be used as targets for antibody-directed lethal photosensitization of infections caused by different MRSA strains. We are now pursuing alternative targeting strategies to improve the killing of MRSA and to reduce the difference in susceptibilities between strains. Indeed, with the selection of a suitable target and photosensitizer combination, targeted lethal photosensitization could be used to prevent and treat infections caused by a variety of bacterial species.

Acknowledgements

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