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Photodynamic therapy for *Staphylococcus aureus* infected burn wounds in mice

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Abstract

The rise of multiply antibiotic resistant bacteria has led to searches for novel antimicrobial therapies to treat infections. Photodynamic therapy (PDT) is a potential candidate; it uses the combination of a photosensitizer with visible light to produce reactive oxygen species that lead to cell death. We used PDT mediated by meso-mono-phenyl-tri(*N*-methyl-4-pyridyl)-porphyrin (PTMPP) to treat burn wounds in mice with established *Staphylococcus aureus* infections. The third degree burn wounds were infected with bioluminescent *S. aureus*. PDT was applied after one day of bacterial growth by adding a 25% DMSO/500 μ M PTMPP solution to the wound followed by illumination with red light and periodic imaging of the mice using a sensitive camera to detect the bioluminescence. More than 98% of the bacteria were eradicated after a light dose of 210 J cm^{-2} in the presence of PTMPP. However, bacterial re-growth was observed. Light alone or PDT both delayed the wound healing. These data suggest that PDT has the potential to rapidly reduce the bacterial load in infected burns. The treatment needs to be optimized to reduce wound damage and prevent recurrence.

Introduction

The growing resistance of pathogenic microorganisms against antimicrobial agents has generated a search for alternative treatments for localized infections. In particular, the use of topical antibiotics is controversial since it has been suggested that such an approach induces antibiotic resistance faster than the use of oral antibiotics.^{1–3} A potential alternative may be antimicrobial photodynamic therapy (PDT),⁴ which is the photoinactivation of microorganisms by reactive oxygen species produced by a light activated photosensitizer (PS).^{5–6} Much is already known about the photodynamic inactivation of microorganisms: both antibiotic-sensitive and -resistant strains can be successfully photoinactivated,^{7–10} and there is the additional advantage that repeated photosensitization of bacterial cells does not induce a selection of resistant strains.¹¹ Superficial wound infections are potentially suitable for treatment by PDT because of the ready accessibility of these wounds for both topical delivery of the PS and light. Burns are particularly susceptible to infection due to the

destruction of the cutaneous barrier and the fact that coagulated and denatured proteins present in the burn provide a nutritious environment for bacterial growth. In humans, burn wound infections have a high morbidity and can impede wound closure.¹²⁻¹⁴ Gram-positive bacteria and in particular *S. aureus* are early colonizers of burn wounds which makes the occurrence of multidrug resistant *S. aureus* a worrisome development.¹⁵

In vivo studies concerning antimicrobial PDT for wound infections are scarce, which is probably due to the requirement of a more complicated experimental set-up.¹⁶⁻¹⁸ In this report we investigated *in vivo* PDT of third degree thermal burn wounds in mice with established bioluminescent *Staphylococcus aureus* infections. To monitor the efficacy of the PDT in real time we used an optical method based on the luciferase mediated bioluminescence emitted by the bacteria. For the experiments described in this article a complete bacterial *lux* operon was integrated into the chromosome of *S. aureus*¹⁹ that allowed the bacteria to emit light without any added substrate for the luciferase enzyme. The emitted bioluminescence was detected *in vivo* with a sensitive imaging camera.²⁰ This method has been validated in several animal studies where bioluminescence intensity has been directly correlated with bacterial CFU numbers from homogenized tissue samples,^{19,21} and it has been shown that the luminescence generated in an infected wound model can be followed for several weeks.²²⁻²⁴ This methodology has the ability to provide information on the size and intensity of the infection in living animals in real time. Due to its noninvasive nature individual animals can be repeatedly imaged and each animal therefore acts as its own control, which dramatically reduces the number of animals needed to attain statistically significant results.

The PS used was 5-phenyl-10,15,20-tris(*N*-methyl-4-pyridyl)-porphyrin chloride (PTMPP), a cationic porphyrin that has proved to be an effective and versatile PS.²⁵⁻²⁸ We investigated the feasibility of the use of PDT as an antimicrobial therapy for infected burn wounds and the effects it has on wound healing. We also compared PDT with silver sulfadiazine cream, which is the standard clinical treatment for infected burns.²⁹ This preparation combines a sulfonamide antibiotic with silver ions that denature protein and prevent DNA replication.^{30,31}

Methods

Bacteria

The bioluminescent mouse pathogenic *S. aureus* strain 8325-4 (XEN 8.1) was created by introducing a modified *Photobacterium luminescens lux* operon using the gram-positive *lux* transposon plasmid pAUL-Atn4001 *lux*ABCDE Km^r²⁷ and was provided by Xenogen Corp (Alameda, CA). Samples stored at -80 °C were thawed and grown in brain-heart infusion medium, in an orbital shaker (37 °C; 100 rpm) to an optical density of 0.6 at 650 nm that corresponds approximately to 10⁸ cells mL⁻¹. The bacteria were washed and resuspended in PBS without Ca²⁺/Mg²⁺ to a density of 10⁸ cells mL⁻¹ for the *in vitro* experiments and 10¹⁰ cells mL⁻¹ for the *in vivo* experiments.

Photosensitizer

5-Phenyl-10,15,20-tris(*N*-methyl-4-pyridyl)porphyrin chloride (PTMPP) was obtained from Buchem Holding BV (Apeldoorn, The Netherlands). Stock solutions of 1 mM were prepared in PBS without Ca²⁺/Mg²⁺ and stored at 4 °C in the dark.

Light source

All illuminations were carried out with 635 ± 15 nm light from a non-coherent light source with interchangeable filtered fiber bundles (LumaCare, London, UK).

***In vitro* studies**

Suspensions of the luminescent bacteria (10^8 CFU mL^{-1}) were diluted 10-fold in PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ or in a 25% dimethyl sulfoxide (DMSO)/PBS mixture; 1 mL aliquots of the diluted cell suspensions were transferred to wells in a 24 well plate. Upon addition of the PS, the samples were illuminated with a fluence rate of 10 mW cm^{-2} , and immediately after the appropriate light dose had been delivered the cell survival was determined. For this, cell suspensions were serially 10-fold diluted with PBS. Of each dilution 10 μL was plated onto square brain–heart infusion agar plates according to the method of Jett *et al.*³² After incubation in the dark at 37°C for 24 h the number of CFUs was counted. Survival was expressed as a percentage of a control sample that contained neither PS nor received illumination. Other control conditions were: illumination in the absence of PS, or incubation with PS in the dark.

***In vivo* studies**

All animal experiments were approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital and were in accordance with NIH guidelines. Male BALB/c mice (20–30 g) were depilated on their backs by use of Nair (Carter-Wallace, New York, NY). The mice were anesthetized by an intraperitoneal injection of a ketamine–xylazine cocktail (90 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine) before burn creation, subsequent PDT, and imaging procedures. Burn wounds were induced by pressing the ends (1 cm^2 area) of two pre-heated brass rods against opposite sides of a raised dorsal skin fold for 10 s.³³ The brass rods were pre-heated in boiling water to about 95°C and produced a third degree burn of $1\text{--}1.5 \text{ cm}^2$ area (1.5–2.2% of body surface area calculated according to Meeh's formula³⁴). Immediately after burning the mice were resuscitated with an intraperitoneal injection of 1.5 mL sterile saline. After ten minutes, 100 μL of the bacterial suspension (10^{10} cells mL^{-1}) was applied to the surface of the wound. The mice were imaged 5 min, 1 h and 24 h after the application of the bacteria (data not shown). PDT was performed 24 h after the burn wound application when the bacteria had been allowed to multiply in the wound and establish an infection. The mice were given a single dose of ketamine/xylazine anesthesia that in our hands lasts for at least 2 h. The PTMPP concentration used was $500 \mu\text{M}$ and the solvent was PBS or a 25% DMSO/PBS mixture, 100 μL of the PS was applied both topically and injected under the burn. The wounds were illuminated directly after the application of the PS with a fluence rate of 84 mW cm^{-2} . The total illumination time was 42 min (short PDT), which gives a delivered light dose of 211 J cm^{-2} , or 84 min (long PDT), which results in a delivered light dose of 423 J cm^{-2} . The mice were imaged before PDT, during interruptions in light delivery and directly after PDT while remaining anesthetized. Furthermore, the mice were imaged on the first three days after treatment and on every other day thereafter until the infection had disappeared. Control groups consisted of mice with infected burn wounds that were subsequently illuminated in the absence of PS, mice with infected burns treated with PTMPP but kept in the dark or mice with infected burns that received no treatment at all. An additional group of mice with infected burn wounds was treated with a 1% silver sulfadiazine cream (Thermazene, Kendall Co, Mansfield, MA) on every day during the infection. Another group was first treated with PDT and thereafter treated with 1% silver sulfadiazine cream application every day until the infection cleared.

Bioluminescence imaging

The low-light imaging system (Hamamatsu Photonics KK, Bridgewater, NJ) has been described elsewhere in detail.³⁵ In short, it consists of an intensified CCD camera mounted in a light-tight specimen chamber, fitted with a light-emitting diode, a set-up that allowed for a background gray-scale image of the entire mouse to be captured. In the photon-counting mode, an image of the emitted light from the bacteria was captured using an integration time of 2 min, at a maximum setting on the image-intensifier control module. By

use of ARGUS software (Hamamatsu Photonics KK, Bridgewater, NJ), the luminescence image was presented as a false-color image superimposed on top of the grayscale reference image. The image-processing component of the software calculated the total pixel values from the luminescence images of the infected wound area. The infection time was defined as the time during which any bioluminescence was present in the wound when measured at the most sensitive setting.

Wound healing

The dimensions of the wounds, width and length, were measured with Vernier calipers on the day of treatment and on the following three days and the areas calculated. From thereon they were measured every other day until the wounds had healed.

Statistics

All experiments were performed at least in triplicate. Values are expressed as means – standard deviation. Comparisons between means of multiple groups were analyzed by one way ANOVA and Bonferroni correction.

Results

In vitro photoinactivation studies

The bioluminescent *S. aureus* could be readily photoinactivated *in vitro*, 0.6 J cm^{-2} (1 min illumination time) of $635 \pm 15 \text{ nm}$ light in the presence of $1.56 \mu\text{M}$ PTMPP was sufficient to obtain $3.9 \log_{10}$ units reduction in viability (Table 1). With this PS concentration no dark toxicity was observed after 10 min. The absorption spectrum of PTMPP shows that the $635 \pm 15 \text{ nm}$ light is absorbed by the Q-bands (Fig. 1).

In vivo PDT studies

One hour after application of the bacteria to the wound no or little luminescence could be observed (data not shown). The applied number of bacteria (10^9 cells per burn wound) was, however, sufficient to induce a reproducible established infection, as could be observed by the degree of luminescence in the wounds 24 h after application (data not shown). This inoculum gave a sufficiently bright luminescence signal from the wound to allow more than $2 \log_{10}$ units of signal reduction to be accurately monitored. The data depicted in Fig. 2 demonstrate that $500 \mu\text{M}$ PTMPP in PBS had only a small light dose dependent effect on reducing the luminescence signal. By contrast, with PTMPP dissolved in the mixture of 25% DMSO/PBS, a total light dose of 211 J cm^{-2} resulted in a decrease in luminescence of almost $2 \log_{10}$ units, compared to only a 70% reduction obtained with the PBS solution and 211 J cm^{-2} . 25% DMSO/PBS had no dark toxicity on the *in vitro* survival of the bacteria; neither did it increase the photodynamic inactivation (data not shown). All further *in vivo* experiments were conducted using the 25% DMSO/PBS mixture as solvent. Fig. 3 shows a typical example of a set of bioluminescence images demonstrating the light dose dependent reduction of the luminescence signal after application of the $500 \mu\text{M}$ PTMPP in 25% DMSO/PBS. No dark toxicity or effect of light alone was observed (data not shown).

Fig. 4a shows the follow up in days of the control groups of mice; PS without illumination, illumination without PS and no treatment at all. Some significant ($p < 0.05$) differences were observed between the different groups during the course of the infection. The luminescence signal in the no treatment group differed significantly from the illumination only group on day 12 ($p = 0.013$) and from the dark group on day 2 ($p = 0.030$) (Fig. 4a). The luminescence of the no treatment group dropped the fastest and the luminescence signal had disappeared after $15.3 (\pm 1.15)$ days. For the dark group this happened after $18.3 (\pm 2.52)$ days and for the illumination only group after $18.7 (\pm 1.15)$ days. Only the difference in

infection time between the no treatment group and the light only group was significant ($p = 0.02$).

The follow-up of the treated groups is shown in Fig. 4b. The luminescence signal in the long PDT group differed significantly from the silver sulfadiazine group ($p = 0.031$) and the combined silver sulfadiazine/PDT treatment group ($p = 0.020$) on day 3. No significant ($p > 0.05$) difference was observed in the total infection time between the groups that had received either short (211 J cm^{-2}) or long PDT (423 J cm^{-2}). For this reason both PDT groups were combined into one PDT group in Fig. 4c and 5.

Fig. 4c shows the total time of infection of all the studied groups. The infection disappeared the earliest in the group of mice that had received the silver sulfadiazine cream. The luminescence signal had disappeared after $13.0 (\pm 5.57)$ days. For the group of mice receiving PDT this was after $18.5 (\pm 5.65)$ days and for the group of mice that had received both PDT and silver sulfadiazine cream the luminescence signal disappeared after $21.3 (\pm 4.16)$ days. These differences were not significant ($p > 0.05$).

The healing of the burn wounds of all the groups studied is depicted in Fig. 5. The wound size of the PDT group differed significantly: from the dark group on day 18 ($p = 0.017$), from the no treatment group on days 12, 16 and 18 ($p = 0.040, 0.047$ and 0.045 , respectively), from the silver sulfadiazine group on days 12 ($p = 0.045$) and 18 ($p = 0.024$). The wound size of the dark group differed significantly: from the no treatment group on days 6 and 14 ($p = 0.034$ and 0.039 , respectively), from the silver sulfadiazine group on day 6 ($p = 0.032$) and the combined silver sulfadiazine/PDT treatment group on days 8 till 16 ($p = 0.007, 0.036, 0.018, 0.027$ and 0.043). The wound healing of the no treatment group differed significantly: from the light only group on days 6 and 12 ($p = 0.045$ and 0.009 , respectively) and from the combined silver sulfadiazine/PDT treatment group on days 12, 14 and 16 ($p = 0.011, 0.015$ and 0.021 , respectively). The wound size of the light only group differed significantly: from the silver sulfadiazine group on day 6 ($p = 0.043$), from the combined silver sulfadiazine/PDT treatment group on days 12, 16 and 18 ($p = 0.041, 0.024$ and 0.034 , respectively). The wound size of the dark group differed significantly from the combined silver sulfadiazine and PDT treatment group on day 12 ($p = 0.017$).

For the group of mice that received PTMPP but was kept in the dark, the group that received no treatment at all and the group that was treated with silver sulfadiazine cream, complete healing was achieved after $22.7 (\pm 2.3)$, $22.7 (\pm 1.2)$ and $19 (\pm 4.6)$ days, respectively. For the group of mice that received PDT, PDT in combination with silver sulfadiazine cream and the group that received illumination alone, complete healing was achieved after $28.8 (\pm 3.6)$, $26.7 (\pm 2.3)$ and $27.7 (\pm 2.9)$ days, respectively. As shown in Fig. 5b, some of these differences were significant ($p < 0.05$). We divided the 6 groups of mice into two groups: group A consisting of mice that had not received any illumination: the dark control, the no treatment and the topical silver sulfadiazine treatment groups *versus* group B consisting of mice that had received illumination: illumination only, PDT and the PDT/topical silver sulfadiazine treatment groups. Group B needed 6.2 days more to obtain wound healing than group A, this difference was significant ($p < 0.001$).

Discussion

The clinical value of PDT as a topical antimicrobial treatment depends both on its bactericidal activity and its cytotoxicity towards host tissue. In this study we have demonstrated that it is possible to rapidly photoinactivate *S. aureus* when present in a burn wound with PTMPP as PS. The fact that the bacteria had a period of 24 hours to colonize the wound and multiply many-fold gives the experiments more clinical significance than if PDT

is carried out a short time after bacterial contamination. The use of stable bioluminescent bacteria allows the progress of the infection to be followed over time in individual mice. It has previously been shown that the bioluminescence from imaging infections in rodents correlates well with CFU counts from homogenized tissues removed from euthanized animals.^{19,21}

The results clearly demonstrate the importance of the solvent used to deliver the PS. When PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$ was used to deliver the PS to the infected wound, a limited reduction in luminescence is observed after PDT. When a 25% DMSO/PBS mixture was used, a substantial fluence-dependent reduction of the luminescence signal was obtained. DMSO is a dipolar aprotic solvent that enhances drug penetration through the skin.³⁶ It is incorporated in a commercial preparation of idoxuridine which is used to treat herpetic infections of the skin. DMSO has also been used topically to treat systemic inflammation, although currently it is only used to treat animals.³⁶ It has been studied for PDT in order to increase the penetration of aminolevulinic acid.^{37,38} We propose that in the present model DMSO permitted the PS to penetrate to the bacteria located at deeper levels in the full thickness skin burn. Alternatively the effect of DMSO may have been to disaggregate the PS molecules and to increase the PDT efficiency of the PS. However, *in vitro* experiments showed no increase in photodynamic inactivation by PTMPP in the presence of DMSO (data not shown). The necessity of using a much higher concentration of PTMPP *in vivo* (500 μM in burn wounds compared to 1.6 μM that efficiently killed bacteria *in vitro*) may be explained by the much higher concentration of biological material in the infected burn (host proteins and cells in addition to bacteria) to which the PS can bind. In addition it was also necessary to use much more light *in vivo* than *in vitro* (211 or 423 J cm^{-2} compared to 0.6 or 1.5 J cm^{-2}); this is also likely to be a reflection of the fact that the bacteria are probably less than 0.001% by weight of the burn lesion. This means that the vast majority of the singlet oxygen produced in the burn infection is wasted on non-bacterial matter and it is therefore necessary to produce much more singlet oxygen to achieve bacterial cell killing. In the *in vitro* experiments where the PS is known to bind to the bacteria and there are no competing cells or proteins present, the photoinactivation is orders of magnitude more efficient. Therefore in order to optimize PDT of burn infections, selectivity of the PS for the bacteria over mammalian cells and proteins is vital.

In all mice studied the luminescence reappeared at the first, second or third day after PDT in various degrees of strength (data of individual mice are not shown but this trend is illustrated in Fig. 4). This is a strong indication that we did not succeed in killing all bacteria present in the burn wound. The anatomy of the burn wound is a perfect environment for bacteria to grow. The denatured tissue is not perfused and can therefore not be reached very well by the immune defense system. The coagulated proteins make a nutritious environment for bacteria to grow. Therefore it is not surprising that re-growth was observed after PDT. In an attempt to inactivate all the bacteria present in the burn wound we increased the total delivered light dose from 211 J cm^{-2} (short PDT) to 428 J cm^{-2} (long PDT) (Fig. 4b). During the first two weeks, the luminescence signal was lower in the long PDT group as compared to the short PDT group. However, this difference was not significant and on day 20 of the infection the luminescence signals were identical. The group treated with silver sulfadiazine cream showed a sharp decrease in luminescence signal after seven days, this demonstrates that this treatment is effective, but not in the early days. Further work with PDT in this burn model should examine the effect of repeating the PDT (dye and light) every two days or similar interval until the natural healing process has advanced to the stage when the host response can cope with the bacteria.

We originally hypothesized that a combination of PDT followed with topical silver sulfadiazine treatment would be successful since PDT would have an immediate effect on

bacterial reduction and the silver sulfadiazine cream could prevent rapid re-growth. Disappointingly this was not the case; the infection lasted up to 21.3 (± 4.16) days with the combined therapy, which is the longest infection period observed for all groups studied (Fig. 4c). A somewhat similar trend can be observed in the wound-healing data (Fig. 5b).

Light alone also exerted a delaying effect on the wound healing (Fig. 5b). When all the groups of mice we studied were divided into two groups, one group that had received illumination and the one that had not, the group that received illumination needed 6.2 days more to heal than the dark group. This suggests that most if not all of the delay in wound healing observed in the PDT treated groups of mice might have been due to illumination damage. The delay in wound healing that we observed due to illumination alone was surprising. Although it is still a controversial topic, the stimulation of wound healing by red light is under investigation. Overall in these studies a total light doses in the order of 1–30 J cm⁻² is used.³⁹ The literature available on the effect of PDT on wound healing does not report negative effects in the control light only groups.^{18,35,40,41}

In previous reports we have shown that topical PDT of wound infections with poly-L-lysine chlorin(e6) conjugates and red light is highly effective in reducing the bacterial burden in excisional wounds as judged by bioluminescence imaging. For a non-pathogenic strain of *Escherichia coli* we showed that PDT eliminated the bacteria and treated wounds healed as well as control wounds.³⁵ For a highly pathogenic strain of *Pseudomonas aeruginosa* we were able to show that when infected wounds were treated with PDT, mice were saved from death by preventing sepsis that occurred in mice with infected wounds left untreated or treated with PS alone or light alone.¹⁸ In this case PDT treated wounds healed better than wounds treated with the topical antimicrobial, silver nitrate solution. This difference in wound healing was attributed to PDT mediated destruction of extra-cellular virulence factors secreted by the bacteria and responsible for delaying wound healing. When soft-tissue infections in mice caused by *S. aureus* were treated by injection of the poly-L-lysine chlorin(e6) conjugate again there was an efficient reduction in bacterial bioluminescence but in this case bacterial re-growth was sometimes observed in the treated area on succeeding days.⁴² The only previous report on the use of PDT to treat burn infections by Orenstein *et al.*¹⁷ used a mixture of deuteroporphyrin and hemin. They showed that the porphyrin mixture was effective in killing *S. aureus* in the burn in the dark without illumination, and regrowth of bacteria was observed 24 hours later.

The recurrence of the infection in the present case together with the delay observed in healing of burn wounds may be due to PDT mediated damage to host tissue as well as the infecting bacterial cells. Because the porphyrin we used is a small molecule it may be relatively quickly absorbed by the mouse cells in the burn wound. Illumination in the presence of PTMPP could result in direct host cell death caused by reactive oxygen species or indirect cell death caused by the impairment of the microcirculation and the inflammatory responses.^{40,43-45} This may have deleterious effects on the cells responsible for wound healing and/or lead to a further deterioration of the wound. We performed PDT one day after the application of the burn wound. It is possible that damage of the granulation tissue is responsible for the delay in healing. It is also possible that *S. aureus* has particular characteristics in terms of virulence factors and pathogenicity that make it susceptible to re-growth after PDT of localized infections. This can be tested by carrying out PDT of burn wounds infected by other microbial species.

There are many possible avenues to explore in order to maximize the efficacy of the therapy. Firstly the PTMPP absorption band is small (extinction coefficient of approximately 8000 at 640 nm) in the red wavelength region of the spectrum that penetrates tissue (see Fig. 1). This necessitated the use of a relatively large fluence of light (429 J cm⁻²) to reach the endpoint

of bacterial reduction in this model. It is possible that an antimicrobial PS with a much higher absorption in the red would have probably worked significantly better. Secondly it is challenging to ensure that a sufficiently high concentration of PS reaches all the bacterial cells in the infected area. The positive effect of adding DMSO to the PS solution suggests that penetration could be a limiting factor and injection of the PS beneath the burn eschar also improved the outcome. Thirdly the problem of bacterial regrowth after PDT would need to be addressed. Although application of the topical antimicrobial silver sulfadiazine after PDT did not prevent regrowth, it is possible that systemic antibiotics would help to prevent recurrence of infection. Another possibility is the use of multiple PDT applications but there has as yet been no study of multiple applications of antimicrobial PDT to infections *in vivo*. It might be expected that the toxicological consequence of multiple applications in patients would depend on the selectivity of the PS for bacteria over mammalian tissue and also on the depth of the infection into the tissue. Relatively superficial infections should be easy to treat multiple times without damaging the host tissue.

In conclusion, our experiments indicate that PDT could be used for the treatment of burn wound infections. The PDT mediated reduction of the bacterial numbers in the burn wound is rapid (less than one hour) compared to a standard therapy (silver sulfadiazine cream) that takes several days to work. Bacterial re-growth after PDT is observed. Targeting of the PS to the bacteria might prevent this and should also exert a tissue sparing effect. We observed that illumination alone exerted a damaging effect. Reduction of the light dose could therefore be beneficial to the wound healing process. This can be accomplished by optimizing the PS absorption coefficient in the red light area. The current clinical practice with patients suffering from third degree burns is now to carry out excision of the burn eschar down to healthy tissue at an early time point.⁴⁶ Although these wounds produced by full thickness excision can still be come infected, the infection would presumably not be as deep as was seen with the present experimental model, and therefore PDT of such infections would be more effective, needing a lower total fluence to give satisfactory bacterial killing. It is not expected that pain that can be caused in patients after PDT to skin lesions using ALA-induced protoporphyrin IX (PPIX) would be a major problem for burn infections using cationic PS. This is because nerve endings are largely destroyed in third degree burns, and it is thought that PPIX has a tendency to accumulate in nerve endings that would presumably not occur with bacterial targeted cationic PS. Burn wound infections appear to be a more complex challenge for PDT than infected excisional wounds.

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Fig. 1.
Chemical structure and absorption spectrum of 10 μ M PTMPP in PBS.

Fig. 2.

Effect of *in vivo* PDT on the bioluminescence of infected burns using 500 μ M PTMPP dissolved in either PBS ($n = 3$) or 25% DMSO/PBS ($n = 5$). Mice were imaged after the marked fluences had been delivered and bioluminescence intensities calculated. Illumination parameters: 635 ± 15 nm, 84 mW cm^{-2} .

Fig. 3.

A typical example of the luminescence reduction of the infected burn wound upon illumination with 635 ± 15 nm, 84 mW cm^{-2} in the presence of $500 \mu\text{M}$ PTMPP in 25% DMSO/PBS. Images were captured after: A: 0, B: 30, C: 60, D: 106, E: 202 J cm^{-2} fluences had been delivered.

Fig. 4.

A: The course of the burn wound infections in the control groups, no treatment ($n = 3$), 500 μM PTMPP without illumination ($n = 3$), no PTMPP with illumination ($n = 3$). Illumination parameters: $635 \pm 15 \text{ nm}$, 84 mW cm^{-2} . B: The course of the burn wound infections in the treated groups, topical silver sulfadiazine (AgSD) ($n = 3$), PDT 428 J cm^{-2} ($n = 4$), PDT 211 J cm^{-2} ($n = 3$), PDT plus topical silver sulfadiazine ($n = 3$). Illumination parameters: $635 \pm 15 \text{ nm}$, 84 mW cm^{-2} . C: The total burn wound infection period in days. Lines connect the groups that significantly differ from each other ($p < 0.05$).

Fig. 5.

A: The burn wound healing course of the burn wounds: no infection ($n = 3$), infected no treatment ($n = 3$); infected 500 μM PTMPP without illumination ($n = 3$); infected no PTMPP with illumination ($n = 3$); topical silver sulfadiazine (AgSD) ($n = 3$); infected PDT 428 + 211 J cm^{-2} ($n = 5$); infected PDT plus topical silver sulfadiazine ($n = 3$). Illumination parameters: $635 \pm 15 \text{ nm}$, 84 mW cm^{-2} . For clarity reasons the error bars were omitted. B: Complete burn wound healing in days. Lines connect the groups that significantly ($p < 0.05$) differ from each other.

Table 1

In vitro photodynamic inactivation of *S. aureus* in the presence of 1.56 μM PTMPP in PBS ($n = 3$).
Illumination parameters: 635 ± 15 nm, 10 mW cm^{-2}

Illumination time/min	Delivered energy/ J cm^{-2}	Log_{10} (reduction in viability)	Standard deviation
0	0	0.0	0.0
1	0.6	3.9	0.3
2.5	1.5	4.8	0.7