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# Can surgical site infections be treated by photodynamic therapy?

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## LETTER TO THE EDITOR

In the previous issue of *PDPDT* [1], the editor, Prof Keyvan Moghissi raised some interesting points relevant to the question of whether antimicrobial photodynamic therapy (PDT) could ever be used as a clinical treatment for surgical site infections. We should like to answer some of the questions raised in the editorial and make some comments that may shed more light on this intriguing possibility.

A surgical site infection was described as a “suppurating wound” with a complex milieu containing host tissue components (skin cells, muscle cells and structural proteins), live and dead bacteria, live and dead leukocytes (principally neutrophils but also containing macrophages and other immune cells), and the exudate, a protein rich fluid containing a variety of molecules including those that help combat the infection, but also containing molecules that potentiate bacterial growth and damage tissue. If the aim of PDT in this complex environment is to kill the bacteria by photochemically generated reactive oxygen species, the issue of selectivity of the photosensitizer (PS) for binding to the microbial cells versus binding to the multitude of other components (both cellular and proteinaceous) that comprise the infection site described above, becomes crucial. In our experience when we compared the PDT dose (molar concentration of PS and J/cm$^2$ of light) necessary to give a certain number of logs of bacterial killing in vitro and also in vivo in an animal infection model, we found that at least one hundred times more PDT is needed in vivo compared to in vitro [2]. What are the reasons for this large difference? We believe that the most important factor is the likelihood that the PS will bind to some of the biological components in the infection other than the bacteria, thus dramatically reducing the availability of the PS to bind to and kill the bacteria. Recall that the actual mass of bacteria in an infection is very small compared to the actual mass of the host tissue (cells and protein). An infection is defined as at least $10^5$ bacteria per gram of tissue and this number ($10^5$) of bacterial cells has a mass of less than 1 μg (a ratio of 1 million:1) [3]. Even if the infection is 1000 times more severe ($10^8$ CFU/g tissue) the ratio is still 1000:1. Another factor to be considered is the ability of the bacteria to penetrate and invade the tissue, meaning that that the PS which is topically applied (see below) may have difficulty in penetrating into the infected tissue to reach bacteria beneath the surface. A third reason is the reduced penetration of the light into tissue due to absorption and scattering, considering in vitro antimicrobial PDT is usually carried out in optically clear media (saline).



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We used two different antimicrobial PS in a relevant infection model; an established soft-tissue infection or abscess caused by *Staphylococcus aureus* in the mouse thigh muscle [4]. A conjugate between poly-L-lysine and chlorin(e6) (pL-ce6) that has a high affinity to bacterial cells and a low affinity for mammalian cells was compared with the free chlorin(e6) that has similar affinity for both bacteria and mammalian cells but can kill Gram-positive bacteria such as *S. aureus* in vitro, after illumination with similar efficacy to pL-ce6. Both PS were injected into the infected area beneath the skin and illuminated with the same red light, but the conjugate produced much more bacterial killing as measured by bioluminescence imaging than did the free ce6. Moreover PDT with the free ce6 produced more damage to the host tissue as judged by loss of the muscle function of the infected leg than did the bacterial-targeted pL-ce6. These data do support the idea that high selectivity of the PS for bacteria over host cells is actually important when real infections are treated.

Another way to ensure that the PS binds as much as possible to microbial cells and as little as possible to host cells is to deliver the PS directly into the infected area by topical application to skin or mucous membranes, instillation into a hollow organ, or by local injection into an abscess or an area of cellulitis. Although PDT for cancer treatment works well after intravenous injection, where the PS molecules pass naturally from capillaries into the tumor cells that rely upon the vessels for nutrition (partly because the tumor capillaries are more leaky than those of normal tissue), it is apparent that this approach would be counterproductive for infections where the destruction of capillaries and the host cells directly supplied by them is unwanted. The requirement for local PS delivery means that certain specialized approaches for drug delivery may be needed. For instance it may be necessary to use specialized surfactants [5] and penetrating agents [6] to help the PS pass into and through the infected tissue. Alternatively physical/mechanical means such as microneedle arrays [7], high-pressure sprays (needle-free injection systems) [8], iontophoresis [9], or fractional laser ablation [10] may be able to increase the penetration of the PS into the infected tissue.

Another important strategy to maximize selectivity of antimicrobial PDT for microbial cells over mammalian host cells is to keep the incubation time short, in other words commence the light delivery soon after delivering the PS into the infected area. The binding of cationic PS to microbial cells with their negative charges is a rapid process (electrostatic binding) and is complete within a few minutes. By contrast the rate of uptake of cationic compounds into mammalian cells occurs by the process of adsorptive endocytosis and is a relatively slow process (several hours). Hence if the incubation time (drug light interval) is prolonged there will be less bacterial killing and more tissue damage.

The issue was raised of whether there is any evidence that PDT for infection could be counter-productive by killing host leukocytes that are fighting the infection rather than the microbial cells themselves. A recent paper does shed some light on this question. Tanaka et al. [11] used a rat model of bacterial arthritis caused by injecting $10^6$ CFU of methicillin resistant *S. aureus (*MRSA) into the knee joint. They then administered Photofrin systemically by IP injection (regardless of the considerations outlined above) and illuminated the knee joint after 24 h. They found that the PDT treatment actually caused the number of bacteria to increase rather than decrease and they attributed this unfortunate finding to the PDT-mediated killing of neutrophils that could therefore no longer control the infection by phagocytosing the bacteria. They confirmed this hypothesis with some in vitro experiments that showed that neutrophils had a high uptake of Photofrin. However it is likely that these investigators would have obtained more favorable results with a different PS that showed selectivity for bacteria over host cells combined with a local injection into the joint rather than a systemic injection.

The last and possibly most important question is what PS should be employed in any clinical trial of PDT for SSI. Although we have presented evidence that obtaining selectivity for

microbial cells compared to host cells is very important, it may not be possible to use this type of PS clinically. Polycationic conjugates such as those using poly-L-lysine [12] or polyethylenimine [13] do exhibit high levels of microbial selectivity, yet these polymers have not attracted much clinical use despite having been widely used experimentally for multiple potential medical applications (gene delivery [14] and cancer drug delivery [15]). They have been thought to have toxicity that precludes their pharmacological use, although this may not be important in local delivery applications. The recent regulatory approval [16] in multi-national arenas for antimicrobial PDT for periodontitis suggests that the "old-fashioned" PS, methylene blue may be the way to go [17]. Generally accepted as safe for local and systemic use (pharmaceutical grade solution is available in every pharmacy) and used for IV injection as a treatment for methemoglobinemia, it has moderate to good efficacy as an antimicrobial PS capable of killing Gram-positive, Gram-negative bacteria and fungi. Toluidine blue is more effective but is less well-explored clinically, and other phenothiazinium PS (dimethylmethylene blue and new methylene blue) are even better but also less well studied [18]. Recent reports [19,20] that the antimicrobial PDT efficacy of these phenothiazinium dyes can be potentiated by inclusion of a bacterial multi-drug resistance pump inhibitor in the PS solution points to a way to increase their usefulness. One exception is the tetra-butyl derivative of toluidine blue (PP904) that has passed toxicology screening under the aegis of Photopharmica in Leeds. It is in clinical trials for PDT of infected non-healing leg ulcers [21].

Despite the above considerations that may limit the overall high activity of antimicrobial PDT in clinical infections, we feel on balance that the time is ripe for clinical trials for infections such as SSI to be initiated.

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